DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES (ESBL) AND METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN CHRONIC SUPPURATIVE OTITIS MEDIA (CSOM) IN A TERTIARY CARE HOSPITAL

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CERTIFICATE

This is to certify that this dissertation entitled **"DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES (ESBL) AND METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN CHRONIC SUPPURATIVE OTITIS MEDIA (CSOM) IN A TERTIARY CARE HOSPITAL"** is the bonafide original work done by **Dr. A.V. KAVITHA,** Post graduate in Microbiology, under my overall supervision and guidance in the Department of Microbiology, Govt. Kilpauk Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

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DECLARATION

I solemnly declare that this dissertation "**DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES (ESBL) AND METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN CHRONIC SUPPURATIVE OTITIS MEDIA (CSOM) IN A TERTIARY CARE HOSPITAL"** is the bonafide work done by me at the Department of Microbiology, Govt. Kilpauk Medical College and Hospital, Chennai, under the guidance and supervision of **Dr. RADHIKA KATRAGADDA, M.D.,** Professor & H.O.D of Microbiology, **Dr. THYAGARAJAN RAVINDER, M.D.,** Professor, Department of Microbiology and **Dr. K.V. LEELA, M.D.,DGO.** Associate Professor of Microbiology Department of Microbiology, Govt. Kilpauk Medical College, Chennai-600 010. This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of Degree of M.D. Branch IV Microbiology examinations to be held in April 2015.

Place: Chennai.

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ABSTRACT

TITLE: DETECTION OF EXTENDED SPECTRUM BETALACTAMASES (ESBL) AND METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)IN CHRONIC SUPPURRATIVE OTITIS MEDIA(CSOM) IN A TERTIARY CARE HOSPITAL

INTRODUCTION:

Chronic suppurative otitis media (CSOM) is one of the most commoninfections of the middle ear which can lead to extra cranial complications and intracranial complications, especially in developing countriesif not diagnosed early. So, early identification and detection of the etiological agent and its sensitivity pattern helps to prevent the complications. Judicial use of antibiotics helps to prevent the emergence of ESBL and MRSA in CSOM.

AIMS AND OBJECTIVES:

1. To isolate and to identify aerobic bacteria from Chronic Suppurative Otitis Media (CSOM) cases.
2. To find out the antibiotic susceptibility pattern of the bacterial isolates.
3. To detect the Extended Spectrum Beta Lactamases (ESBLs) producers from Gram negative bacterial isolates.

4. To find the prevalence of Methicillin Resistant Staphylococcus aureus (MRSA) among Staphylococcus aureus isolates.

MATERIALS AND METHODS:

Total number of cases included in the study were two hundred and twelve Ear discharge were collected using the sterile cotton wool swabs.Direct gram staining was done&pusculture and bacterial identification was done. Antibiotic susceptibility testing was performed by the Kirby -Bauer disc diffusion method on MHA (Mueller Hinton agar) according to CLSI guidelines. Detection of ESBL producers was done by Phenotypic Confirmation Test Minimum Inhibitory Concentration (MIC) of Bacterial Isolates was performed by agar dilution method. Detection of Metallo beta lactamase (MBL) producers was done by Combined disc test with Ethylene diamine tetra acetic acid ((EDTA).AmpC was detected by AmpC disc test and Ceftazidime- Imipenem Antagonism test (CIAT). MRSA was detected by cefoxitin disc diffusion method.

RESULTS:

Out of 212 cases of CSOM, 157 cases (74.06%) were culture positive and 55 (25.94%) were culture negative .Among the 157 culture positive isolates, Gram negative organisms constitutes 113 (65.32%) and Gram positive organisms constituted 60 (34.68%) of the cases. The common age group suffering from CSOM were in the age group between 21 to 30 years 55

(25.94%) followed by 49(23.11%) in the age group OF 31 to 40 years. Males 110 (51.88%) were more affected than females 102(48.11%), Observations in this study indicates male preponderance in cases of CSOM.

Pseudomonas aeruginosa 80 (46.24%) was found to be the most commonly isolated bacteria among the Gram negative isolates. In our study Staphylococcusaureus 46 (26.59%) was the most common gram positive organism isolated.

Pseudomonas aeruginosa77(96.25%) was sensitive to amikacin and 76(95%) sensitive to ofloxacin. Klebsiellapneumoniae 20(80%) were sensitive to amikacin. All the Gram negative bacterial isolates showed 100% sensitivity to imipenem and piperacillin/tazobactum except Pseudomonas aeruginosa78(97.50%). Extended spectrum beta lactamases (ESBL) producers isolated from Gram negative bacteria causing CSOM was 10(30.30%).

Among the Staphylococcus aureus isolated, 41 (89.13%) and 40(86.95%) were sensitive to amikacin and ofloxacin respectively. Staphylococcus aureus isolates were 100% sensitive to vancomycin and linezolid. 9(19.56%) of Staphylococcus aureus isolates were Methicillin resistant Staphylococcus aureus (MRSA). All the 9(19.56%) of MRSA were 100% sensitive to vancomycin and linezolid.

All the 10 isolated ESBLs were confirmed by combined disc test and Minimum Inhibitory Concentration (MIC) reduction and also were subjected to PCR.

1(16.66%) was found to be MBL producers by Ceftazidime- EDTA method,but none of them were found to be MBL producing by imipenem–EDTA method.

By AmpC disk test and CIAT, 3(2.65/%) were AmpC producers.

CONCLUSION:

Among the Gram negative organisms, Pseudomonas aeruginosa 80 (46.24%) was found to be the most commonly isolated bacteria followed by Klebsiellapneumoniae 25 (17.09%)in our study. Staphylococcusaureus 46 (26.59%) was the most common gram positive organism isolated. Among the Staphylococcus aureus, 9 (19.56%) were found to be MRSA AmongEnterobacteriaceae isolates 10 were found to be ESBL producers.

Thus, it is essential to detect Extended Spectrum BetaLactamases(ESBL)and Methicillin Resistant Staphylococcus aureus (MRSA) routinely in laboratories. Administration of antibiotics should be prescribed cautiously to prevent the emergence of bacterial resistance in the hospital and community.

Key words: Chronic Suppurative Otitis Media, Pseudomonas, ESBL, MIC, MRSA

INTRODUCTION

Chronic suppurative otitis media (CSOM) is one of the most common infections of the middle ear which can cause extra cranial complications and intracranial complications and includes morbidity. It is one of the main causes of preventable hearing loss, principally in the developing world[1]. CSOM is believed to develop in early childhood, often followed by poorly managed acute otitis media, with its potential spilling into adulthood which accounts for recurrent episodes of chronic discharging ears that can last for many years.

In 1996, WHO/CIBA Foundation workshop had defined Chronic suppurative otitis media as a stage of disease in which there is chronic infection of the middle ear cleft, i.e., eustachian tube, middle ear and mastoid and in which a non-intact tympanic membrane (e.g., perforation or tympanostomy tube) and discharge (otorrhoea) are present for at least 2 weeks or more[2].The basic feature is the presence of a non-intact tympanic membrane which is common to all the cases of chronic suppurative otitis media[3].Around 90% of the burden is borne by the countries in the Africa, South-east Asia and Western Pacific regions and some ethnic minorities present in the Pacific Rim. CSOM is rare in the Americas, Australia, Europe, and the Middle East. Amongst the South-East Asian countries,

prevalence of India (7.8%) is high. This is a recent estimate from a survey conducted in a school in Tamil Nadu and is lower than earlier estimates which ranged from 16% - 34%[1].

CSOM is persistent and destructive disease resulting in irreversible sequelae leading onto serious intra and extra cranial complication[4]. Conductive or acquired hearing loss is worldwide, especially in children, and particularly in developing countries. In both children and adults, CSOM infections can lead to prolonged hearing loss which in turn not only affects the developmental impairment of linguistic, behavioural, motor and social skills but also the performance at school or work place[5].

Typically, viral infection of the upper respiratory tract precedes the disease, but soon the conditions are favourable for the middle ear to be invaded by pyogenic organisms[4]. The middle ear infection can spread to important structures such as, facial nerve, mastoid, labyrinth, lateral sinus, meninges and brain which can lead to facial nerve paralysis, mastoid abscess, labyrintitis, lateral sinus thrombosis and meningitis[6].

Pseudomonas aeruginosa is a common organism causing chronic suppurative otitis media. Over the past few years, various researchers have isolated Pseudomonas from 48-98% of patients diagnosed as CSOM. From chronically discharging ears, the second most common organism isolated is Staphylococcus aureus. Reported data estimate infection rates from 15-30% of culture-positive draining ears. Large variety of gram-negative organisms causes the rest of the infections. Klebsiella (10-21%) and Proteus (10-15%) species are slightly more common than other gram-negative organisms[7].

CSOM has been given significant attention, not only because of its increasing incidence and, but also chronicity due to ototoxicity with both topical and systemic antibiotics and bacterial resistance [5].

The development of multiple resistant strains of bacteria which can produce both primary and postoperative infections is due to the wide spread use of antibiotics. Persistence of low grade infections is the result of the indiscriminate, haphazard and half-hearted use of antibiotics and poor follow-up of patients. The introduction of sophisticated synthetic antibiotics has led to the changes in the microbiological flora and also increased the significance of reappraisal of the present day flora in CSOM.

The in vitro antibiotic sensitivity pattern is very essential for the clinician to plan a general outline of treatment for a patient with a chronic discharge in the ear[3, 4, 6].

Many patients with discharging ears use antibiotics before they approach the hospital .This alter the bacterial flora. This may lead to development of resistance to bacteria to antibiotics leading to treatment failure and persistence of discharge in the ears. It is therefore essential to know the bacteria causing suppurative otitis media and sensitivity which enables to initiate the treatment with specific antibiotics and also to prevent complications[8].

The present study aims to find out the bacteriological profile, their antimicrobial susceptibility pattern and to detect Extended Spectrum Beta Lactamases (ESBL) production and Methicillin Resistant Staphylococcus aureus (MRSA) among the bacterial isolates causing Chronic Suppurative Otitis Media.

AIMS AND OBJECTIVE

1. To isolate and to identify aerobic bacteria from Chronic Suppurative Otitis Media (CSOM) cases.
2. To find out the antibiotic susceptibility pattern of the bacterial isolates.
3. To detect the Extended Spectrum Beta Lactamases (ESBLs) producers from Gram negative bacterial isolates.
4. To detect Metallo Beta Lactamases (MBL) producers from non-fermenters.
5. To detect AmpC betalactamases from Gram negative bacterial isolates.
6. To find the prevalence of Methicillin Resistant Staphylococcus aureus (MRSA) among Staphylococcus aureus isolates.

REVIEW OF LITERATURE

The history of middle ear infection was found to be present as early as 460 B.C, when Hippocrates (460-377 BC) noted that acute ear pain with continued strong fever is to be dreaded, for the patient may become delirious and die[8].The discovery of cholesteatoma in a skull found in Norfolk, UK by McKenzie and Brothwell (1967) which were thought to be of Anglo-Sexon date[9].In 1829, Curveilhier described cholesteatoma as 'pearly tumours' which was later accepted as the general term 'cholesteatoma'. In 1854, Cholesteatoma was described by Virchow in detail. Evidence of previous infections in the middle ear was obvious when radiological evidence in the mastoid have been the subject of inquiry in 417 temporal bones from South Dakota Indian burials and in 15 prehistoric Iranian temporal bones[10,11].

EPIDEMIOLOGY OF CSOM

Disease report burden released by the National Commission of Macroeconomics and Health enumerates common disease conditions in India. Major diseases in group of maternal ,communicable, and perinatal conditions includes as follows HIV/AIDS, diarrheal diseases, malaria and

other vector borne disease, leprosy, childhood disease, and otitis media. Throughout the world, the burden of illness due to CSOM comprises of 65–330 million individuals with ear discharge, out of them, 60% suffer from significant hearing loss. CSOM constitutes about 28,000 deaths and about 2 million DALYs. The burden of illness is around 90% seen mostly in countries such as Africa, South-east Asia and Western Pacific regions and some ethnic minorities in the Pacific Rim. CSOM is infrequent in the Americas, Australia, Europe, and the Middle East[1]. Otitis media leads to loss of 0.47 million disability adjusted life years (DALYs) and causes 0.1% of share in the total burden of disease in India[12]. A study conducted at Himachal Pradesh by Amar Singh et al[13] reported that CSOM was the common diagnosis among the ear diseases in rural India which constitutes about 32.75%. In the study conducted by Kiran Gaur et al[12], the prevalence of CSOM was 19.2%.Amongst the countries in South-East Asia, Thailand has prevalence rates which ranged from 0.9 to 4.7% whereas the prevalence in India was found to be high(7.8%) which was a recent estimate from a survey conducted in a school in Tamil Nadu and is lower than earlier estimates which ranged from 16% - 34%.According to Rupa et al, the overall prevalence rate of CSOM among Rural South Indian children was found to be 6%[1].

RISK FACTORS OF CSOM

Risk factors for the chronic suppurative otitis media includes crowded conditions, high rate of cross infection leading onto prolonged carriage of nasopharyngeal pathogens, early nasopharyngeal acquisition of otological pathogens, malnutrition and age at first episode of acute otitis media[2].

The rate of CSOM is higher among people with poverty, illiteracy, ignorance, poor hygiene and malnutrition, poor housing, lack of breast feeding, impaired immunologic status, passive smoking, frequent URI and lack of medical facilities[14].

The susceptibility of certain races, such as the Australian Aborigines, Alaskan Eskimos, Greenlanders and South-western American Indians to CSOM is also well documented. These are the risk factors which possibly favour the development of CSOM by diminishing the immunological defenses, raising the inoculum, and encouraging earlier infection[1].

PATHOPHYSIOLOGY OF CSOM

CSOM commences with an acute infection. The pathophysiology of this disease initiates with irritation and succeeding inflammation of the middle ear mucosa. Mucosal edema occurs in response to inflammation. Persistent inflammation finally leads to ulceration of mucosa and subsequent epithelial lining breakdown. The host's attempt at bringing down the infection or inflammation establishes as granulation tissue. This can produce polyps inside the middle ear cleft. The sequence of the above changes may continue, which in turn destroys the surrounding bony margins and finally leading to the several complications of CSOM[15].

PATHOGENESIS OF CSOM

Perforation of tympanic membrane occurs usually secondary to acute otitis media. Many investigation reports that healing of tympanic membrane is facilitated by hyperplasia, proliferation and migration of the outer keratinizing squamous epithelium, the absence of which is attributed primarily to persistent infection[2]. The histopathological changes in CSOM vary with the degree and extent of the disease. The middle ear cleft is lined by a single layer of cuboidal or columnar epithelium. The hypotympanum

and the region below the horizontal course of the facial nerve contains goblet cells whereas the region above and behind, the lining cells are flat and devoid of glandular structures.

The following changes are seen in COM without cholesteatoma: development of chronic inflammatory infiltrate which consists of lymphocytes, plasma cells and histiocytes and it is associated with increased capillary permeability of lamina propria of the middle ear mucosa and mucosal edema. The middle ear epithelium undergoes transformation resembling respiratory epithelium which consists of increased number of goblet cells and ciliated cells. The epithelium becomes glandular. This change also takes place in the mastoid air cells and in the middle ear cavity. The secretion from newly formed gland is an important part of the discharge seen in CSOM. Decrease in the vascularity and fibrosis are the characteristic feature of the late stage of the disease. These changes are particularly seen in mastoid air cells in which sclerosis and new bone formation occurs. Tympanosclerosis is a special form of fibrosis often seen in CSOM[16].

In both types of CSOM, erosion of the ossicular chain occurs. Erosion is due to overproduction of cytokines – interleukin -2, platelet derived factor, fibroblast growth factor and TNF alpha which in turn

promote hypervascularisation, resorption of osteoclast and bone resulting in damage of ossicles. Middle ear infection is more harmful the nearer it is to the ossicular chain and also when it is present for a longer period[17].

Pseudomonas aeruginosa attaches itself with the help of pili to necrotic or diseased epithelium of the middle ear. After attachment, proteases, lipopolysaccharide, and other enzymes are produced by the organism to prevent immunological defenses. The damage produced by enzymes of bacteria and inflammation results in further damage, necrosis, and bone erosion which can lead to some of the complications of CSOM. Serious complications or disseminated disease rarely occur in the normal immunocompetent individual[15, 18].

CLASSIFICATION OF CSOM

CSOM is principally categorized into two types: Tubotympanic otitis media is disease condition which affects the pars tensa. When the pars flaccida part of the tympanic membrane is affected, it is known as atticoantral type [3, 4, 6].

TYPES OF CHRONIC SUPPURATIVE OTITIS MEDIA:

Chronic suppurative otitis media is classified as two types:

1. Tubotympanic disease (safe type)
2. Atticoantral disease (unsafe type)

TUBOTYMPANIC DISEASE[2, 19]

Since it does not cause much serious complications, this is also called as safe disease. The infection is restricted to the antero inferior part of the middle ear cleft and the mucosa. In this type, there is no risk of erosion of bones. If present, the discharge will appear through a perforation in the pars tensa part of the tympanic membrane. The rim of remnant ear drum or intact annulus surrounds the perforation (Central perforation).Usually, the shape of the perforation is kidney shaped due to the limited blood supply to the affected portion of the ear drum.

ATTICOANTRAL DISEASE[20]

The atticoantral type is also known as unsafe type because it is associated with complications. They are associated with granulation tissue and cholesteatoma which may be attic or postero superior. Cholesteatoma is characterized by accumulation of keratinizing stratified squamous

epithelium in the middle ear or pneumatised part of temporal bone. It may be present along with infection. Cholesteatoma causes bone erosion and necrosis which affects the important structures such as facial nerve, inner ear, ossicles leading to hearing loss and intracranial complications.

MICROBIOLOGY OF CSOM

In CSOM, aerobic (e.g. Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis, Klebsiella species) or anaerobic (e.g. Bacteroides, Peptostreptococcus, Proprionibacterium) bacteria or both may be the causative agent. The bacteria are occasionally present in the skin of the external auditory canal, but they may multiply in the presence of high humidity, inflammation, trauma or lacerations. These bacteria may enter the middle ear through a chronic perforation in the membrane[2].

GRAM NEGATIVE BACILLI	Pseudomonas aeruginosa
	Klebsiella pneumoniae
	Proteus spp.
	Escherichia coli
	Enterobacter spp.
	Citrobacter spp.
	Acinetobacter spp
GRAM POSITIVE COCCI	Staphylococcus aureus
	Coagulase Negative Staphylococcus aureus (CONS)
	Enterococcus faecalis
	Streptococci spp
ANAEROBES	Anaerobic Gram positive cocci
	Bacteroides spp.
	Clostridium spp.
	Prevotella spp
FUNGI	Aspergillus flavus
	Aspergillus niger
	Aspergillus fumigatus
	Candida spp.

References from Shasidhar Viswanath et al[21], and WHO report (2004).

Many studies have implicated in CSOM has found that Pseudomonas aeruginosa is the most common bacteria isolated from CSOM. The toxins and enzymes produced by Pseudomonas aeruginosa have been particularly responsible for the deep-seated and progressive destruction of middle ear and mastoid structures. Pseudomonas aeruginosa is an opportunistic extracellular pathogen which is frequently seen in chronic infection. To invade the host system two mechanisms are known. One of them is by producing large number of extracellular products. The other mechanism is by production of biofilm[2].

The most commonly isolated organism from the CSOM is Pseudomonas aeruginosa. 48-98% of patients with chronic ear discharge are due to Pseudomonas sps in several studies done earlier.

The second most common organism isolated from chronically draining ears is Staphylococcus aureus. According to earlier data reports, 15-30% of culture-positive draining ears account for infection rates. Remaining infections is due to various gram-negative organisms. Compared to other gram-negative organisms, Klebsiella (10-21%) and Proteus (10-15%) species are more common.

In 5-10% of cases, infections are polymicrobial, commonly with a mixture of Staphylococcus aureus and Gram-negative organisms. The remaining spectrum of colonizing organisms responsible for this infection are Fungi (Aspergillus, Candida) and anaerobes (Bacteroides, Peptostreptococcus, and Peptococcus) of which anaerobes constitutes20-50% of the isolates causing CSOM. They are tend to be associated with cholesteatoma. In about 25% of cases, Fungi have been isolated, but their contribution to pathogenesis of this disease is unclear[7].

Biofilms are complex bacterial groups that attach to the implanted biomaterial or mucosal surfaces and are embedded in a slim-like extracellular matrix containing polysaccharides, nucleic acids and proteins known as extracellular polymeric substances (EPS). Since they have efficient defense mechanisms against the host's immune system and against antimicrobial substances, they are difficult to eradicate. The importance of biofilms in otolaryngologic infections is becoming increasingly apparent Pseudomonas aeruginosa is thought to occur as a biofilm in chronic suppurative otitis media, and in cystic fibrosis (CF) infection of the airway[22].Lee et al[23] stated that frequency of biofilms was 60% (6 of 10) in CSOM. According to Lampikoskiet al[24], the frequency of biofilms was 66%

(19 of 29) in mastoid mucosa with CSOM. Therefore, Roland suggested that biofilms are the probable causative agent of CSOM, which gives the explaination of the observed antibiotic resistance[25].Ercan Kaya et al found that the presence of biofilms was significantly higher in patients with CSOM (70%).the biofilm provides an almost an impervious layer to many systemic and topical antibiotics and may increase the resistance to antibiotics from 20- 100 times. Mature biofilm protects pathogenic microbes from antimicrobial treatment by providing a physical barrier, protecting the bacteria from phagocytosis and immune proteins. Microbes within biofilm also appear to have a lowered metabolic activity, providing further protection from antimicrobial treatment. Bacteria are intermittently released from the biofilm creating chronic or recurrent local and systemic infection.

BACTERIAL RESISTANCE[26]:

Resistance of microorganisms to drugs involve many mechanisms which are as follows:

1. Certain microorganisms produce enzymes which destroy active drug. For ex. β lactamases produced by staphylococci and gram negative organisms.
2. Some of them change their permeability to the drug

3. Developing an altered target for the drug or altered metabolic pathway which bypasses the reaction inhibited by the drug.
4. Production of an altered enzyme that can perform its metabolic function but is much less affected by the drug.

ORIGIN OF DRUG RESISTANCE:

Non genetic:

Microorganisms that are not metabolically active may be phenotypically resistant to drugs, but their offspring are fully susceptible. They can also be resistant by losing the specific target for a drug for several generations or they can infect the sites where the antimicrobials are not active

Genetic:

Most of the drug resistant microbes evolve as a result of genetic change. Chromosomal resistance develops due to spontaneous mutation in a locus which controls susceptibility to a given antimicrobial drug. Extra chromosomal resistance occurs through plasmids which carry genes for resistance to more than one drug.

LABORATORY DIAGNOSIS OF CSOM:

Laboratory diagnosis of CSOM includes routine blood investigation, microscopical examination of direct gram stain, culture and sensitivity of ear discharge, PCR to detect bacterial DNA.

SAMPLING COLLECTION METHODS[8]:

To collect the specimens in chronic suppurative otitis media for the purpose of culture and sensitivity, different methods have been utilised which include

1. Cotton wool swabs are used.
2. Discharge from external auditory meatus are collected
3. Aspiration of discharge with the help of needle from the middle ear (tympanocentesis)
4. Suction aspiration of the discharge from the middle ear through the tympanic membrane perforation.

Though tympanocentesis increase the specificity of the culture but it loses its value when the discharge from the middle ear reaches the external auditory canal following perforation of the tympanic membrane.

The identification of causative organisms is lost when the discharge mixes with the preexisting flora present in the external auditory meatus. In the present situation in India, both in rural and urban area there is a lack of

health services. Therefore in majority of cases, patients reach the ENT departments only after the discharge has appeared in the external auditory canal. In such situations, the ear discharge in the external auditory canal is collected with the help of cotton wool swabs or by collecting the discharge in a test tube or container for culture and sensitivity and it has to be relied by the clinician. This may not be a proper indicator of the causative agents in all the cases. Since the middle ear aspiration suction technique, involves the collection of discharge directly from the middle ear space through the non-intact tympanic membrane it remains to be more sensitive when it is compared to the two methods describes above. In middle ear suction aspiration method, external contamination is very little because sterilized apparatus is used. Atleast 48 hours before sample collection, already prescribed antimicrobials/topical antibiotic drops were stopped since these can transfer organisms from the external auditory meatus to the middle ear space that can change the bacteriological picture.

BETA LACTAMASES

BETA LACTAM ANTIBIOTICS

Beta lactam antibiotics are the ones which contain β lactam ring in its structure. It includes Penicillins, Cephalosporin, Monobactams and Carbapenem[27].

MECHANISM OF ACTION[28]

All beta lactam antibiotics inhibit the bacterial cell wall synthesis. The bacteria produce UDP- N – acetyl muramic acid and UDP – N – acetyl glucosamine. The peptidoglycan synthesis residues are linked together forming long strands and the UDP is split off. Transpeptidases cleaves the terminal D alanine of the peptide chain. The cross linking is necessary for the strength and rigidity of the cell wall. The beta lactam antibiotics inhibit the transpeptidases so that cross linking does not take place.

BETALACTAMASES

This is a heterogeneous group of penicillin recognizing proteins. They belong to members of super family of active site serine protease. These enzymes inactivate β lactam antibiotics[27].

CLASSIFICATION OF BETA LACTAMASES[29]

Schemes of functional classification that were accepted by β-lactamase researchers include:

(i) In 1968, Cephalosporinases and penicillinases were grouped on the basis of reaction to specific antibody (Sawai et al[30]).

(ii) In 1973, the Richmond and Sykes scheme classified the enzymes into five main divisions based on the substrate profile and the gene coding for β-lactamase.

(iii) In 1989, Bush scheme classified β-lactamase on the basis of molecular structure and the substrate inhibition.

(iv) In 1980, Ambler was the first to propose the Molecular structure classifications.

(v) More recently, Bush, Jacoby, and Medeiros devised a classification scheme based on the sequence of nucleotide on the genes for placing β-lactamases into functional groups and on the enzyme's biochemical properties and molecular structure.

CLASSIFICATION OF BETA LACTAMASES[29]

(Classification Schemes for Bacterial Beta Lactamases)

Bush-Jacoby-Medeiros group	1989 Bush group	Richmond-Sykes class	Mitsuhashi-Inoue type	Molecular class	Preferred substrates	Inhibited by:		Representative enzyme
						CA[b]	EDTA	
1	1	Ia,Ib,Id	Csase[a]	C	Cephalosporins	-	-	AmpC from Gram negative bacteria, MIR-1
2a	2a	not included	Pcase V	A	Penicillin	+	-	Penicillinases from Gram Positive bacteria
2b	2b	III	Pcase I	A	Penicillins, Cephalosporins	+	-	TEM-1, TEM-2, SHV-1
2be	2b'	not included except K1 in class IV	Cxase	A	Penicillins, Narrow spectrum and extended spectrum Cephalosporins, Monobactams.	+	-	TEM-3 to TEM-26, SHV-2 to SHV-6, *Klebsiellaoxytoca* K1
2br	not included	not included	not included	A	Penicillins	±	-	TEM-30 to TEM-36, TRC-1
2c	2c	II,V	Pcase IV	A	Penicillins,Carbenicillins	+	-	PSE-1, PSE-3, PSE-4
2d	2d	V	Pcase II, Pcase III	D	Penicillins, Cloxacillin	±	-	OXA-1 to OXA-11, PSE-2 (OXA-10)
2e	2e	1c	Cxase	A	Cephalosporins	+	-	Inducible cephalosporinases from *Proteus vulgaris*
2f	not included	not included	not included	A	Penicillins, Cephalosporins, Carbapenems	+	-	NMC-A from *Enterobacter cloacae*, Sme-1 from *Serratia marcescens*
3	3	not included	not included	B	Mostβ lactams, including carbapenems	-	+	L1 from *Xanthomonasmaltophilia* CcrA from *Bacteroides Fragilis*
4	4	not included	not included	ND[c]	Penicillins	-		Penicillinase from *Pseudomonas Cepacia*

a Csase, cephalosporinase; PCase, penicillinase; CXase, cefuroxime-hydrolyzing b-lactamase.

b CA, clavulanic acid.

c ND, not determined.

DETECTION OF BETA LACTAMASES[27, 31]

Biochemical tests are used to detect β lactamases. β lactamases hydrolyses benzyl pencillin to produce penicilloic acid which was measured. This acid production was measured by

1. Acidometric method: change in pH of an indicator dye is measured.
2. Iodometric method:
3. Chromogenic cephalosporin method:

Normally Nitrocefin is yellow in colour which turns red on production of β-lactamases**.**

B LACTAMASE INHIBITORS[27]

Beta lactamases inhibitors are enzymes that are similar to β lactam antibiotics. They bind either reversibly or irreversibly to beta lactamase, thereby preventing β lactam antibiotics from destruction. They act as suicide bombers, utilizing all available enzymes. Clavulanic acid, Sulbactam and Tazobactam are some of the examples of beta lactamases that has gained importance in clinical medicine.

EXTENDED SPECTRUM BETA LACTAMASES (ESBL)

THE OCCURRENCE OF ESBLS

ESBLs are β-lactamases that belong to Ambler class A. They have the ability to produce resistance to the first-generation cephalosporin, second-generation cephalosporin and third-generation cephalosporin, penicillins and aztreonam (but not the carbapenems or cephamycins). These antibiotics are hydrolysed by these enzymes. β-lactamase inhibitors such as clavulanic acid inhibit these enzymes[32] and placed under Bush's classification 2be[33].

ESBL's are first reported in Germany in 1983 followed by France in 1985 among Klebsiella spp. ESBL occurs in every part of the world and in most genera of enterobacteriaceae[32]. Generally, ESBLs are β-lactamases which are plasmid mediated. They are present most commonly in Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis and other Gram negative bacilli[34]. There are more than 200 different ESBLs have been reported[33].

Before the development of first β-lactam, penicillin, the resistance to β-lactam antibiotics was emerged. Chromosomal mediated β-lactamase was found naturally in many genera of gram negative bacteria. These

enzymes are believed to have developed from penicillin-binding proteins and show some sequence homology with them. The development was probably due to the selective pressure exerted by Beta-lactam-producing soil organisms that are present in the environment[35].

The first plasmid-mediated β-lactamase, TEM-1, was described in the early 1960s in gram-negative bacteria. The TEM-1 enzyme was first reported in a strain of Escherichia coli isolated from a blood culture of the patient named Temoniera in Greece.

TEM-1 is plasmid and transposon mediated, hence its spread is rapid in other species of bacteria. Within a short period after its first isolation, worldwide spread of the TEM-1 β-lactamase has spread throughout the world. Presently, it is found in Gram negative bacteria. SHV - 1(for sulphydryl variable) the next common plasmid mediated β-lactamase is found in Escherichia coli and Klebsiella pneumoniae. In majority of K. pneumoniae isolates, it is chromosomal mediated whereas it is plasmid mediated in E. coli[35].

CTX-M β lactamases, one of the most important ESBLs was discovered in Escherichia coli strains which was isolated from patients in Germany in 1989. Over the past few years, Cefotaximase (CTX-M) was

found in several genera of Enterobacteriaceae particularly in Escherichia coli and Klebsiella pneumoniae. Cefotaxime, which is a broad spectrum cephalosporin, is efficiently hydrolysed by the CTX-M enzymes which can also be inhibited by β lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam. There are over 60 different subtypes of CTX-M identified. On the basis of their amino acid sequences, they have been grouped under five different clusters - CTX-M-I, CTX-M-II, CTX-M-III, CTX-M-IV, CTX-M-IV[36]. Over the past few years, CTX-M type has become a global problem amid the clinical isolates of the family Enterobacteriaceae. Hospital outbreaks of these enzymes have been reported in various countries of the world[37].

The emerging role in the acquisition of drug resistance is the gene transfer among bacteria and the main cause of resistance transmission is known to be the horizontal dissemination of resistance genes[38]. Irrational use antibiotic use is also a risk factor for acquiring an ESBL-producing organism. It was found that there was a strong relationship present between acquisition of ESBL producing strain and use of third generation cephalosporin[32]. Plasmids that code for ESBL enzymes may also carry co-resistant genes for other non-beta lactam antibiotics like aminoglycosides[32,33].

PREVALENCE OF ESBLs IN INDIA

In India, the prevalence of ESBLs has reached epidemic proportions, which ranges from 62% - 100% in Klebsiella spp and Escherichia coli which was obtained from infection of skin and soft tissue, blood stream infections and respiratory infections as seen in the 10 Indian medical centre SENTRY study[39].Generally, the rate of ESBL was 84% in SENTRY surveillance taken in India. As part of the SARI (2004–2006) study[40], out of the 163 Gram negative isolates tested the prevalence of ESBL was 88%.

In India, a study was conducted in nine centres, each representing different cities in several regions of India: NewDelhi, Mumbai, Bangalore, Chennai, Tamil Nadu, Hyderabad, Kolkata, Lucknow and Indore, ESBL producing isolates were observed in high rates which indicates that ESBL producers are not only found throughout the country but to every single city or region. In addition to TEM and SHV ESBL types, isolates from India also produces CTX-M enzymes[39].

Presently, ESBLs are rising as major problem for patients not only in hospitals but also in long-term care facilities and community. These bacteria have not only become endemic but have caused outbreaks in various hospitals all over the world[32]. Therefore, it is imperative to detect

the ESBL production in clinical microbiology laboratory to guide the clinicians to provide appropriate therapy[33]

METHODS FOR DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES

SCREENING OF ESBL

1. Disc Diffusion method[32,34,41]

The disc diffusion methods are the screening test for ESBL production by Escherichia coli, Klebsiella and Proteus mirabilis as proposed by CLSI 2013.Laboratories use cefotaxime, ceftazidime, cefpodoxime, ceftriaxone and aztreonam for the screening of ESBL production. More than one of these antibiotics should be used to improve the sensitivity of the detection. If the screening test is positive, it should be confirmed by phenotypic confirmatory test.

CLSI Recommendations for ESBL Detection [41]

Drugs(cephalosporins)	CLSI (M100-S23) recommended Zone diameter(sensitive)
Cefotaxime	≥27mm
Ceftriaxone	≥25mm
Ceftazidime	≥22mm
Cefpodoxime	≥17mm
Aztreonam	≥27mm

III. ESBL Detection methods recommended by Clinical Laboratory Standard Institute (CLSI)[41.]

1. Phenotypic confirmatory test/ Disc Potentiation Test[32,34,41]

The CLSI advocates the phenotypic confirmatory test for the detection of production of ESBL by Klebsiella and Escherichia coli which use the cefotaxime or ceftazidime discs (30µg) with or without clavulanate (10µg). Confluent growth of test organism on Mueller Hinton agar shows difference of 5mm along the cephalosporin with clavulanate disc compared to cephalosporin disc alone.

2. Double disc synergy test[32,34,41]

In Mueller – Hinton agar plate a lawn culture of the test organism is done on which third generation cephalosporin disc (Ceftazidime, Cefotaxime or Ceftriaxone) and Augmentin are placed 30 mm apart. When zone of inhibition of cephalosporin extends towards Amoxycillin/clavulanate disc, it is taken as positive for production of ESBL.

3. Broth Micro dilution[32]

Disc potentiation test can also be done using broth microdilution assays by using ceftazidime (0.25to128μg/ml), ceftazidime with clavulanate (0.25/4 to128/4 μg/ml), cefotaxime (0.25 to64μg/ml), and cefotaxime with clavulanate (0.25/4 to 64/4 μg/ml) decrease in MIC of two fold serial dilution of cephalosporin with clavulanate compared to the MIC of cephalosporin alone suggests positive for ESBL production.

4. Minimum Inhibitory Concentration[42]

Minimum inhibitory concentration was performed by agar dilution method. Mueller Hinton agar was prepared in glass petri plates and sterilized by autoclaving which is then cooled to 50 ˚c in a water bath. Third generation cephalosporins like cefotaxime, ceftazidime was prepared

in serial dilutions in sterile distilled water .it was brought to a final concentration ranging from 2µg to 2048µg/ml. at 50 ˚c antibiotics were added to the medium and mixed well which was poured into sterile culture plates. The medium has to be used immediately to retain the potency. For each test, test medium without the antibiotic was prepared and used as control plate.

Media Preparation

Freshly prepared and autoclaved Mueller Hinton agar is allowed to cool in a 50˚c water bath. Preparation of serial dilution of 3rd generation cephalosporin (ceftazidime, cefotaxime) was prepared in sterile distilled water which gives a final concentration of 2µg – 2048µg/ml of agar. The drugs are added to the medium at 50˚c. It is mixed well and poured onto sterile petri plates. Test medium without the antibiotic was used as control plate for each series of test.

Preparation of Inoculum

3-5 well isolated colonies belonging to same morphological type should be taken and mixed in about 4-5 ml of a suitable broth medium in a sterile test tube. At a temperature of 35˚c, the broth culture was kept for

incubation till it attains 0.5 McFarland turbidity medium. This suspension corresponds to150 million organisms/ml.

Test plate inoculation

0.003ml of inoculum was added to appropriate quadrants divided on the plates of various concentrations and incubated at a temperature of 37º c for a time period of 16-20 hrs. Minimum inhibitory concentration is the least concentration at which there is no visible growth. Various concentration of cephalosporin with 4 micrograms/milliliters of clavulanic acid ranging from 0.5µg to 2048µg/ml of agar was tested with isolates and the MIC was obtained.

COMMERCIAL METHODS AVAILABLE TO DETECT ESBL

(i) Epsilometer-Test for ESBLs[43,44]

Plastic drug impregnated strips are produced by AB bio disk in which one end contains a gradient of ceftazidime (MIC test ranges from 0.5µg - 32µg/ml) and with a ceftazidime gradient and constant concentration of clavulanate (4µg/ml). The manufacturer recommends

about eight – fold decrease in MIC in the combination with clavulanate. The sensitivity of this method is reported as 87-100% and the specificity as 100%.

(ii) Vitek ESBL[44]:

Vitek ESBL cards contain cefotaxime and ceftazidime alone and cephalosporin plus constant concentration of clavulanate. Cards are inoculated in the same manner as that for regular Vitek cards. Cards are analysed automatically as soon as the growth in the control well has attained a set threshold. A prefixed reduction in the growth of cefotaxime and ceftazidime plus clavulanic acid containing wells is compared with the growth in the wells having cefotaxime/ceftazidime alone, indicates positive for ESBL producer. The sensitivity and specificity of the test is more than 90%.

(iii) Molecular ESBL detection techniques

Test	Advantages	Disadvantages
DNA Probes	Specific for gene family(e.g., TEM or SHV)	Labour intensive, cannot distinguish between ESBLs and non ESBLs, and between variants of TEM or SHV
PCR	Easy to perform, specific for gene family(e.g., TEM or SHV)	cannot distinguish between ESBLs and non ESBLs, and between variants of TEM or SHV
Oligotyping	Detects specific TEM variants	Requires specific oligonucleotide probes, labour intensive, cannot detect new variants.
PCR- RFLP	Easy to perform, can detect specific nucleotide changes	Nucleotide changes must result in altered restriction site for detection.
PCR- SSCP	Can distinguish between a number of SHV variants	Requires special electrophoresis conditions.
Nucleotide sequencing	Gold standard, can detect all variants	Labour intensive, can be technically challenging, can be difficult to interpret manual methods.
Real Time PCR	Rapid identification, minimum cross contamination	Expensive, technical skill required

METALLO BETA LACTAMASES

Metallo-β-lactamases (MBL's) are carbapenemases produced mainly by Pseudomonas aeruginosa which require zinc at the site of action. They are designated in Ambler's Class B and Bush-Jacoby Medeiros Group 3. They hydrolyze virtually all β-lactam agents such as penicillin, cephalosporin, including the carbapenems[45].

Pseudomonas aeruginosa producing metallo-β-lactamases (MBLs) was reported first in Japan in the year 1991 and subsequently it has been isolated from several parts of the world, including Asia, Australia, Europe, North America, and South America [46].

Till now seven major types of MBL were described worldwide – IMP, SPM, VIM, GIM, SIM, AIM-1 and NDM-1. Among them, *bla*IMP and *bla*VIM are the most common types of MBLs that are prevalent worldwide. From India, only *bla*VIM and NDM-1 have been reported in P. aeruginosa in the past[45].

VIM (Veronese Imipenemase) enzymes have been categorised into three main groups designated VIM-1, VIM-2, and VIM-7. Until now, among Pseudomonas aeruginosa isolates, VIM-2 is spread extensively,

whereas VIM-1 is usually restricted to Enterobacteriaceae isolates at least 27 unique variants are present in the IMP sub lineage which differs by up to 22% amino acid sequence divergence (between IMP-9 and IMP-19) that show important difference in structure and function from each other or from enzymes of other sub lineages. Determinants of IMP-type MBL are usually found in Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter baumannii isolates, though in other organisms (e.g., Pseudomonas putida) they have also been identified sporadically [45].

TESTS TO DETERMINE METALLO BETA LACTAMASES AMONG NON FERMENTERS[45, 46]

SCREENING FOR MBL

An isolate of P. aeruginosa was considered screen-test positive for MBL when it was Imipenem resistant: 10 µg (IPM) and/or Meropenem: 10µg (MEM) and/or Ceftazidime: 30 µg (CAZ). Antibiotic sensitivity was done by the Kirby-Bauer disc diffusion method as per CLSI recommendation.

CONTROLS

P. aeruginosa (blaVIM and blaIMP positive: CMC, Vellore) and ATCC 27853 were used as positive and negative controls in all tests performed.

CONFIRMATION OF MBL PRODUCTION

All screen-test positive isolates were subjected to:

DOUBLE DISC SYNERGY TEST[45]

In this test, Imipenem (10 μg), Meropenem (10 μg), Ceftazidime (30 μg) discs were kept on the Mueller Hinton agar (MHA) plate already seeded with test organism matching 0.5 McFarland (10^8CFU/ml) . The discs are kept with centre to centre distance of 20 mm from a disc containing 5 μl 0.5 M EDTA (930 mg). Plates were incubated at 37°C for 18-20 hours. Enhancement of zone of inhibition around Imipenem and/or Meropenem and/or Ceftazidime toward the EDTA disc when compared with the zone of inhibition of corresponding antibiotic disc was interpreted as positive for MBL production.

COMBINED DISC TEST/DISC POTENTIATION TEST[45]

Two discs each of 10 µg Imipenem, 10 µg Meropenam and 30 µg Ceftazidime were placed on lawn culture of 0.5 McFarland test organism seeded on MHA. To one disk of Imipenem, Meropenem and Ceftazidime, 5 µl of 0.5 M EDTA (930 mg) was added. Plates were kept for incubation at the temperature of 37°C for 18-20 hours. After incubation, inhibition zone was noted. Organisms which showed increased zone of inhibition by 7 mm or more around any or all of the three discs with EDTA or showed increase in 5-28 mm inhibition around only CAZ-EDTA disc as compared to Imipenem, Meropenem and Ceftazidime discs alone, respectively, were considered to be MBL producers. A blank disc of EDTA was tested as control.

The MBL E test procedure[47]

MBL E test strips with IMP (4 to 256µg/ml) and IMPE (1to 64µg/ml) were applied on Mueller-Hinton agar inoculated with test organism. The plates were kept for incubation for a time period of 16 to 20 hrs at a temperature of 35°C. It was reported as metallo β lactamase producer if there is reduction of IMP MIC≤ 3 twofold dilutions in the

presence of EDTA. Similarly, a "phantom" zone is present between the two gradient divisions or distortion of the IP ellipses indicates the presence of metallo-ß lactamases. The MICs were read as resistant, intermediate and sensitive according to CLSI guidelines (Resistant: MIC ≥16 ug/ml, sensitive: MIC≤ 4ug/ml).

MODIFIED HODGE TEST[48]

MHA plate is streaked with the ATCC Escherichia coli 25922 and an imipenem disc is placed in the centre. Imipenem resistant isolates are inoculated from the edge of the disc to the periphery of the plate. It is incubated overnight and read. Imipenem hydrolyzing strains produce distortion on the zone whereas non hydrolyzing zones do not produce any effect.

AMP C BETALACTAMASES

AmpC β-lactamase produced by Escherichia coli was the first bacterial enzyme reported to destroy penicillin. Mutations with stepwise-enhanced resistance were termed *ampA* and *ampB*. Reduced resistance caused by mutation in an *ampA* strain was designated as *ampC.* There had

been many changes in most of the *amp* nomenclature over the years but the designation *ampC* has persisted. The *ampC* gene from Escherichia coli was reported as well as sequenced in 1981.

In the structural classification of β-lactamases by Ambler, AmpC enzymes was designated in class C, whereas in the functional classification scheme by Bush et al, they were designated to group 1.Typically, the molecular masses of AmpC enzymes were 34 to 40 kDa and have isoelectric points of ≥8.0. They are more active on cephalosporins than penicillins. Oxyiminocephalosporins such as cefotaxime, ceftriaxone, ceftazidime and monobactams such as aztreonam, Cephamycins such as cefotetan and cefoxitin are hydrolyzed by these enzymes. Aztreonam, oxacillin and Cloxacillin, however, are good inhibitors. Though Cefuroxime, Cefotaxime, Ceftazidime, Ceftriaxone, Cefepime, Aztreonam and Piperacillin are weak substrates and weak inducers, they will be hydrolyzed if enough enzymes are present[49].

Two types of Amp C are present – Plasmid mediated and Chromosomal. Klebsiella sps, Salmonella sps and Proteus mirabilis are plasmid mediated whereas Citrobacter freundii, Enterobacter cloacae, Hafnia alvei, Morganella morganii, and Serratia marcescens. The unique feature of Escherichia coli is that it expresses chromosomal Amp C at low

levels. Constitutive hyper production or hyperinducibility of AmpC is due to mutation in *ampD* which is the commonest cause of AmpC overexpression[50]

Pseudomonas aeruginosa has three *ampD* gene, production of enhanced AmpC occurs in a stepwise manner. The most common resistance mechanism against various β-lactam drugs is the selection of mutations leading to the hyper production of chromosomal *AmpC*. The derepressed mutants can be selected in clinical settings expressing resistant phenotype. Several such chromosomally mediated *Pseudomonas* – derived cephalosporinases (PDC) with extended-spectrum cephalosporinases activities have been reported among Pseudomonas aeruginosa. In addition to chromosomal *AmpC*, the production of plasmid - mediated *AmpC* represents a new threat in the treatment of infection caused by P. aeruginosa[51].

METHODS TO DETECT AmpC BETA LACTAMASES

AmpC DISK TEST[52]

In a MHA plate, a lawn culture of *E. coli* ATCC 25922 was made. Sterile saline (20µl) was put on the sterile disks (6 mm) which was later

inoculated with several colonies of test organism. Cefoxitin disk (almost touching) was placed on a fresh inoculated plate. The inoculated disk was then placed adjacent to cefoxitin disk and incubated overnight at 35°C. The inference was made as follows:

Flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk is produced, it is taken as positive test. A negative test had an undistorted zone.

Modified Three Dimensional Tests[52]

Fresh overnight growth of the test organism was transferred from Mueller Hinton agar (MHA) to a sterile micro centrifuge tube which was weighed previously and to obtain 10-15 mg of bacterial wet weight, it was weighed again to determine the weight of bacterial mass. The pellet was made by suspending the bacterial mass into the peptone water and centrifuged for 15 minutes at 3000 rpm. The bacterial pellet was repeatedly freeze thawed (approximately 10 cycles) to prepare the crude enzyme extract. Mueller Hinton Agar plates were taken and a Lawn culture of *E. coli* ATCC 25922 was made on it. Cefoxitin (30 μg) disk were placed on the Mueller Hinton Agar plates. Linear slits (3 cm) were cut 3 mm away from cefoxitin disk, using sterile surgical blade. The enzyme

extract was put in a small circular well which was made at the other end of the slit. A total of 30 to 40 µl of extract was loaded in the well at a 10 µl increment. The Liquid in the plates were allowed to dry after keeping it upright for 5 to 10 minutes and were incubated for 24 hrs at 37°C. The presence of AmpC β-lactamases was interpreted and considered a positive three-dimensional test when there is enhanced growth of the organism on the surface at the point where the slit inserted the zone of inhibition of cefoxitin. Three different types of results were recorded according to the zone of inhibition of the cefoxitin.

AmpC producers	-	Clear distortion
Non-AmpC producers	-	No distortion
Indeterminate strains	-	Minimal distortion

CEFTAZIDIME IMIPENEM ANTAGONISM TEST (CIAT) [51, 53]

In this test, a ceftazidime disk (30 µg) is kept 20 mm away (edge-to-edge) from an imipenem disk (10 µg) on a Mueller-Hinton agar plate which was priorly inoculated with bacterial suspension matching with 0.5 McFarland standard. The plate was incubated for 24 hrs at 35°C. A cefoxitin disk was used for comparison was kept 20 mm away from the

ceftazidime disk. Inducible AmpC beta-lactamase production was considered as positive if there is a visible reduction in the zone of inhibition around the ceftazidime disk adjacent to the imipenem or cefoxitin disks is present.

NON BETA LACTAM INHIBITORS[49]

One of the best known Amp C inhibitors is boronic acid. Several boronic acid derivatives have been added to an empty sterile disc. The disk loaded with boronic acid is then placed adjacent to β lactam disc. Yagi et al reported that an increase of ≥5mm in the zone of inhibition around a ceftazidime or cefotaxime disc already loaded with 300μg of 3 amino phenyl boronic acid detects all AmpC varieties. It was negative in coproduction of ESBLs and carbapenemases.

CLOXACILLIN COMBINED DISK DIFFUSION TEST (CCDDT) [54]

The test was performed, to detect both inducible and constitutive AmpC. The test was performed by using cefoxitin and ceftazidime antibiotic disks with and which were placed at a distance from each other. Increase in ≥ 5 mm in the zone diameter for any one of the antimicrobial,

when tested in combination with cloxacillin, when compared with its zone when it was tested without cloxacillin confirms the AmpC β-lactamase production.

DOUBLE DISK SYNERGY TEST (DDST) [54]

The test was performed by using cefotaxime and ceftazidime disks. Between these two discs, a cloxacillin disk was placed at a distance of 10 mm. If there is synergism between cloxacillin and ceftazidime and/or cefotaxime, then it indicates AmpC β-lactamase producers. This test detects both inducible and constitutive Amp C inducers as well.

METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

Staphylococcus aureus is the most important human pathogen present in the external environment and in the anterior nares of 20- 40% of adults. It is also seen in the axillae, intertriginous skin folds, the perineum, and the vagina[27].It is responsible for several infections which ranges from mild skin and soft tissue infections to severe life threatening infections. Originally, penicillin was the drug of choice for the treatment of serious Staphylococcus aureus infections. The advent of penicillin resistance in the

S. aureus was due to the acquisition of plasmid borne genetic elements coding for β lactamase production[27].

Later, penicillinase-resistant, semisynthetic penicillins such as oxacillin, methicillin was the drug of choice for these organisms[27]. Incorrect use of many antibiotics, intravenous drug abuse, long hospital stay, nasal carriage of MRSA is some of the important risk factors for MRSA acquisition[55]. In 1961, the MRSA case was first noticed in the United Kingdom within a short period of introduction of methicillin into clinical practice. In October 1960's, MRSA was reported [56, 57]. The latter type of resistance is due to the manifestation of different penicillin binding protein known as PBP2a which results from the acquisition of a chromosomal gene known as mecA. Strains of Staphylococcus aureus which express the mecA determinant are called as MRSA (Methicillin Resistant Staphylococcus aureus[27].

Infections produced by Methicillin-resistant Staphylococcu saureus (MRSA) account for 40-60% of all nosocomial Staphylococcus aureus infections in many centers across the world. Since 1970s, MRSA was the main cause of healthcare-associated infections. In 1990s, sudden increase in the number of MRSA infections was found in populations without prior

healthcare contact. This increase has been associated with the recognition of new MRSA strains, often called community- associated MRSA (CA-MRSA) strains[58].

MRSA in India

In India, the significance of MRSA was recognized late. It emerged as a major problem in the 80's and in the 90's.the incidence of MRSA in western part of India is about 25% whereas it is 50% in South India. Community Acquired MRSA has been reported more in India[56].

MECHANISM OF RESISTANCE

The chromosomally localized mecA gene is responsible for methicillin resistance. Penicillin binding protein 2a synthesis requires mecA gene. Penicillin binding protein are membrane bound enzyme which catalysis the transpeptidation reaction which is responsible for cross linking of peptidoglycan chains.PBP2a has low affinity for all beta lactam antibiotics .it substitutes other PBPs which enables Staphylococcus to survive exposure to high concentration of beta lactam antibiotics[27].

Four different SCCmec elements have been recognized. SCCmec type I, II and III is associated with Health care associated MRSA (HA-MRSA). Community associated MRSA tend to carry Type IV element.CA-MRSA have been typed as USA 300 and 400 with the help of pulse field gel electrophoresis.CA – MRSA has a characteristic antibiotic susceptibility pattern, carries specific virulence factor such as Panton – valentine leukocidin and specific SCC mec type IV[59].

MRSA DETECTION IN LABORATORY[27]

Staphylococcus aureus is identified by its colony morphology. Gram stain shows the presence of gram positive cocci in clusters .It also gives positive catalase and coagulase test. Phenotypic and genotypic methods are used to detect Staphylococcus aureus.

METHODS TO DETECT MRSA

1. DISC DIFFUSION METHODS[60]

Cefoxitin disc diffusion test

Cefoxitin belongs to cephamycin is a potent inducer of mecA gene. Several studies have reported that cefoxtin disc diffusion test has good results in determining mecA gene than the oxacillin disc diffusion. The test is performed by placing 30µg of cefoxitin disc in the Mueller Hinton Agar plate without NaCl supplementation. The plate is kept in a incubator at the temperature of 37˚c. The zone of inhibition is determined after 24 hrs and the zone size is interpreted as susceptible ≥21mm, resistant ≤21 mm as per CLSI guidelines.

2. Oxacillin screen agar method

Oxacillin screening is done by using 6 mg/ml of oxacillin in Mueller Hinton agar with 4% NaCl. All methicillin resistant strains were confirmed by this method. Plates were incubated at 30˚C. The strains which grow in this medium were considered as Methicillin resistant Staphylococcus aureus (MRSA).

3. MIC determination[61,62]

(i) Agar dilution method

4-5 discrete colonies were emulsified onto 4-5 ml of nutrient broth which is adjusted to 0.5 McFarland standard.0.0001ml is used as the final inoculum. The concentration of oxacillin used is 32µg-0.015µg/ml. After drying, 1µl of inoculum is inoculated in the plates using a calibrated platinum loop. The plates are incubated at 37˚cfor 24hrs.MIC is the lowest concentration at which no visible growth occurs. Susceptible ≤2µg/ml, resistant ≥4µg/ml.

(ii) Broth dilution method

To a Mueller Hinton broth with 4%NaCl, serial dilution of oxacillin is added. Few colonies of Staphylococcus aureus are emulsified into fresh peptone water and adjusted to match 0.5 McFarland standard which is used as inoculum. It is incubated at 33-35° c or 24 hrs. Oxacillin MIC < 2µg/ml, Resistant > 2µg/ml.

(iii) E test

The E test, a quantitative method for testing susceptibility of antibiotic applies for both antibiotic diffusion and dilution into the medium. It contains a thin inert carrier strip which has a predefined stable

antimicrobial gradient present on it. When this E strip is placed in Mueller Hinton agar plate inoculated with test organism, there is prompt release of the drug into the medium. After incubation, a symmetrical inhibition ellipse is produced. The intersection between the carrier strip and the edge of inhibitory zone denotes the MIC value.

(iv) Latex agglutination test for detection of PBP 2a[63]

200µl of extraction reagent is taken into which a loopful of bacterial cells is added. It is then subsequently lysed by boiling for 3 min and cooled to room temperature. To 200µl of lysate 50µ l of another extraction reagent is added and mixed well. It is then centrifuged at 1500rpm.40µ l of supernatant is taken and used as for testing agglutination with sensitized latex particles. The card is rotated for 3 min. the resulting agglutination patterns are read.

NEWER METHODS

Screening test

Mannitol salt agar cefoxitin screening medium[64]

Mannitol salt agar plates with cefoxitin concentration of 2, 3 or 4mg/ml is prepared. Fresh overnight cultures are used to prepare inoculum which matches 0.5 Mc Farland's standard. The test inoculum is streaked

onto the agar and kept for incubation at the temperature of 35 – 37˚ c for 48 hrs. The results are interpreted at 18 to 48hrs. Positive results are taken when there is any visible growth in the medium.

CHROMagar MRSA[65]

It is used for direct identification of MRSA from clinical specimens. When the specimen is applied to the agar, MRSA appears as light mauve to mauve colonies after 16-18 hrs of incubation. Other colonies will grow as white or beige or has poor growth.

GENOTYPIC METHODS

Multiplex PCR for MRSA detection of mec A and fem B genes. Coag genes, ccr genes, nuc genes, toxin genes.

1. Real time PCR

MRSA isolates are detected directly from blood culture bottles using real time PCR assays. Based on melting curve analysis, the assay differentiates into clusters.

2. Pulsed Field Gel Electrophoresis[66,67]

From an overnight grown culture of a single colony, a bacterial pellet is processed and the restriction fragments are separated on the gel. Gel is stained with ethidium bromide. The photo is taken under Ultra violet light. Strain relatedness among CA-MRSA and HA-MRSA isolates can be investigated.

3. Multi locus Sequence Typing(MLST)[66]

The clonal evaluation of MRSA is detected by MLST. Sequential analysis from 7 Staphylococcus aureus shows the housekeeping genes as follows i.e. aroE, arcC, glpF, gmK, pt, tpi and yqil. Each isolate is defined by all the alleles of the seven genes. This results in an allele profile / gene sequence type (ST).

4. Microarray Analysis[68]

Multiplex PCR products can be used as hybridization samples. After hybridization at the test site of the microarray, detection of fluorescence is done automatically by the instrument images of the array. Automatically captured image is then analysed using the image analysis software of the instrument.

MANAGEMENT OF INFECTION WITH ESBL – PRODUCING ORGANISMS

In vitro, cephamycins and carbapenems are sensitive to strict ESBL producers showed little resistance if any inoculum effect with these agents. When the inoculum size is raised from 10^5 to 10^7 organisms, even both Piperacillin / Tazobactam and cefepime shows reduced sensitivity to TEM and SHV type ESBLs producers.

Inspite of the use of a standard inoculum, cefepime is resistant to CTX –M type and OXA type ESBLs strains.

Inhibitor- resistant β lactamases

The tazobactam and piperacillin/tazobactam combination are still sensitive to inhibitor –resistant TEM variants whereas clavulanic acid and sulbactam are resistant to it.

AmpC

AmpC producers are usually resistant to cephamycins and oxyimino-beta lactams but they are sensitive to carbapenems but diminished porin expression makes them resistant to carbapenem as well.

Management of MRSA infections

The drug of choice for serious infections caused by Methicillin resistant Staphylococcus aureus is Vancomycin. MRSA infections can be treated by Vancomycin and Teicoplanin which are glycopeptide antibiotics. Teicoplanin has a longer half-life when compared to vancomycin. For systemic infections, intravenous route is preferred since the glycopeptide antibiotics have poor oral absorption. Due to the inconvenient route of administration of vancomycin, treatment of MRSA infection can be difficult. Many newly discovered MRSA strains shows resistance even to vancomycin and teicoplanin. These are called as Vancomycin Intermediate resistant Staphylococcus aureus. Daptomycin, Linezolid, tigecycline and Quinupristin/Daltopristin are the some drugs used to treat vancomycin resistant infection.

MATERIALS AND METHODS

This cross sectional study was done for a period of one and a half year from January 2013 to August 2014 to study the bacterial isolates from patients with CSOM at Government Kilpauk Medical College and Hospital, Chennai. A total of 212 samples collected from both sexes in all age groups were studied during this period.

INCLUSION CRITERIA

1. Clinically diagnosed Chronic Suppurative Otitis Media cases of various age groups and both sexes were included.

2. Patients who were not on antibiotic (both systemic and topical) treatment for minimum of 1 week prior to sample collection.

EXCLUSION CRITERIA

1. Nonbacterial pathogens causing CSOM.

2. Patients with other middle ear infections were excluded.

3. Patients having discharge for less than 3 weeks.

SAMPLE COLLECTION[69]

Pus samples from ear discharge were collected under aseptic conditions using two sterile cotton swabs after obtaining informed content. The external ear was first cleaned using sterile cotton swabs soaked in sterile physiological saline. After cleaning, the exudates were collected using sterile cotton swabs using aural speculum.

SPECIMEN TRANSPORT

Samples were sent immediately to microbiology laboratory without delay.

SAMPLE PROCESSING

DIRECT GRAM STAIN

One swab was used to do direct gram stain study for the presence of pus cells, epithelial cells and bacteria.

CULTURE

Second swab was used for inoculation of Nutrient agar, 5% Sheep blood agar and Mac Conkey agar. To differentiate pathogens from commensals, standard microbiological methods were followed. The importance of the isolates obtained was assessed on the basis of clinical history, presence of pus cells in direct gram stain, pure growth on culture.

ANTIBIOTIC SUSCEPTIBILITY TESTING[70]

Antibiotic sensitivity testing was performed on Mueller Hinton agar using Kirby Bauer disk diffusion method. Interpretation of the results was done by measuring the sizes of the zone of inhibition according to CLSI guidelines 2013(M100-S23).

Escherichia coli - ATCC 25922.

Pseudomonas aeruginosa - ATCC 27853.

Staphylococcus aureus - ATCC 25923

were used as quality control strains.

TURBIDITY STANDARD FOR INOCULAM PREPARATION

For a susceptibility test, density of the inoculum is standardized by a BaSO4 turbidity standard, which is equal to 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), should be used.

Inoculum preparation

Isolated colonies (approx. 3-5) belonging to similar morphological type should be taken and mixed in about 4-5 ml of a suitable broth medium in a sterile test tube. The broth culture is incubated at 35˚c until it attains 0.5 McFarland turbidity medium. This suspension corresponds to150 million organisms/ml.

Method of inoculation of test plates

The turbidity of the test medium was adjusted and it was inoculated within 15 minutes into the plates. A sterile cotton swab is immersed into the suspension and it is pressed along the sides of the tube to remove the excess inoculum from the swab. The swab is then streaked onto a previously dried Mueller Hinton agar plate. This procedure is repeated 2-3

times by rotating the plate 60˚c so that the inoculum is evenly distributed onto the plate. Finally, the rim of the plate is swabbed. Within 15 minutes, the antibiotic discs are impregnated onto the plate. The discs should be pressed firmly to confirm complete contact with the surface of the agar. It is ensured that the discs are placed 24mm from center to center. The plate is inverted and kept in the incubator within 15 minutes after the application of the drugs. The plate is examined after 16-18 hrs of incubation. The zone of inhibition will be uniformly circular with a confluent lawn of growth if the inoculum was correct. The diameter of the inhibition zone are calculated with the help of sliding calipers or rulers and interpreted as per CLSI guidelines

METHODS TO DETECT EXTENDED SPECTRUM BETA LACTAMASES[32, 34, 41]

Quality control

Quality controls were performed using

Escherichia coli ATCC 25922 - Negative control

Klebsiella pneumoniae ATCC 700603 - Positive control.

Disk diffusion methods

Disk diffusion test was done for all Enterobacteriaceae isolates against cefotaxime (30 μg), ceftriaxone (30 μg),cefpodoxime (10 μg) and ceftazidime (30 μg) antibiotic disks for the screening of the isolates for potential ESBL production. Overnight incubation was done at 37˚c after which the zone size was read as per CLSI recommendations for ESBL screening criteria.

Phenotypic confirmatory tests or disc potentiation test

This test was done for all enterobacteriaceae isolates against ceftazidime (30 μg) antibiotic discs with and without clavulanic acid (10 μg). These discs were placed on a Mueller –Hinton agar plate inoculated with bacterial suspension equivalent to 0.5 McFarland standards. Overnight incubation was done at 37°C after which the result was interpreted as follows:

If the zone diameter of ceftazidime with clavulanic acid was increased ≥ 5 mm when compared with ceftazidime alone was taken as positive for ESBL production.

MIC determination - Agar dilution method

Media Preparation

Freshly prepared and autoclaved Mueller Hinton agar was allowed to cool in a 50 ˚c water bath. Preparation of serial dilution of 3rd generation cephalosporin (ceftazidime, cefotaxime) was prepared in sterile distilled water which gives a final concentration of 2µg – 2048µ g/ml of agar. The drugs are added to the medium at 50˚c.It was mixed well and poured onto sterile petri plates. Test medium without the antibiotic was used as control plate for each series of test.

Preparation of Inoculum

3-5 well isolated colonies belonging to same morphological type should be taken and mixed in about 4-5 ml of a suitable broth medium in a sterile test tube. The broth culture was incubated at 35˚c until it attains 0.5 McFarland turbidity medium. This suspension corresponds to150 million organisms/ml.

Test plate inoculation

0.003ml of inoculum was added to appropriate quadrants divided on the plates of various concentrations and incubated at 37 c for 16-20 hrs. Minimum inhibitory concentration is the lowest concentration at which no visible growth occurs. Various concentration of cephalosporin with 4µg/ml of clavulanic acid ranging from 0.5µg to 2048µg/ml of agar was tested with isolates and the MIC was obtained.

DETECTION OF ESBL PRODUCERS BY POLYMERASE CHAIN REACTION (PCR) [71]

DNA Extraction methods

DNA extraction was done with the help of DNA Purification kit (PureFast Bacterial Genomic DNA purification kit) and polymerase chain reaction master mix.

Constituents of Master Mix 2X

1. Taq DNA Polymerase - 2Units.
2. 10X Taq reaction buffer
3. 2mM Magnesium Chloride.
4. 10mMdNTPs mix - 1µl.
5. Polymerase Chain Reaction additives.

Agarose for the purpose of Gel Electrophoresis - Agarose, 50XTAE buffer,6Xgel loading buffer, Ethidium bromide were used.

Procedure of DNA Extraction

1. Pellet was suspended in 200µl of Phosphate Buffer Saline (PBS).
2. Add 50µl of lysosome and incubated at37 c for 15 min.
3. Mix lysis buffer(400µl) and proteinase K(40µl).
4. Incubate the mixture in a water bath at 70 c for ten minutes.
5. The whole lysate was transferred into pure fast spin column. It is then kept in centrifuge at 10000rotations/min for about 1 min.
6. Discard flow through and 500µl of wash buffer-1 was added and placed in centrifuge for 1 min
7. Discard flow through 0.5ml of wash buffer – 2 and kept in centrifuge for 1min. This procedure was done again and washed for one more time.
8. Discard flow thorough. To remove the residual ethanol column was centrifuged for 2 min
9. Add 100µl of elution buffer to elute DNA and then centrifuged for 1 min.
10. Extracted DNA quality and quantity was checked by loading in 1% agarose gel and 1µl of extracted DNA was used for amplification by PCR.

PRIMERS

CTX-M-14

Product size - 120bp

5'-TTATGCGCAGACGAGTGCGGTG-3'

5'-TCACCGCGATAAAGCACCTGCG-3'

SHV-12

Product size- 276bp

5'-CGCCGCCATTACCATGAGCGAT-3'

5'-ACCCGATCGTCCACCATCCACT-3'

TEM primer

Product size - 148bp

5’-CCAAACGACGAGCGTGACACCA-3’

5’-AGCGCAGAAGTGGTCCTGCAAC-3’

Procedure of Polymerase Chain Reaction

[25microliters of Master mix has 10X Taq buffer, 2mM Magnesium Chloride. 0.4mMdNTPs mix, and 2UProof reading Taq DNA Polymerase]

1. Reaction was done as follows:

   In PCR vial

Components	Quantity
Master Mix	25µl
Forward Primer(3pmoles/µl)	2.5µl
Primer – Reverse(3pmoles/µl)	2.5µl
Genomic DNA	5µl
nuclease free water	18µl
Total volume	50µl

2. Mix and spin down briefly and gently.
3. It was kept into Polymerase Chain Reaction machine and programmed in the following manner:

   Initial denaturation was done at 95 c for 3 minutes.

   Denaturation - 95˚ c for 30 seconds.
   Annealing - 55˚ c for 30 seconds.
   Extension - 72˚ c for 30 seconds.
   } 35 cycles

   Final extension - 72˚c for 5 minutes.

Loading

1. Prepare 2% agarose gel. [2grams of agarose was added to 100ml of 1x TAE buffer]
2. 8µl of 6X Gel loading dye is added to each PCR vial and 5 microliters of PCR sample is loaded.
3. Electrophoresis is done at 50V until the dye reaches $3/4^{th}$ of distance and look for bands in Ultraviolet Transilluminator.

PREPARATION OF AGAROSE GEL ELECTROPHORESIS

1. Preparation of 2% agarose was done as follows. 2grams of agarose is mixed with 100ml of 1X TAE buffer and it was liquefied by heating in a microwave oven.
2. Around 60ºC, 5µl of Ethidium bromide was added to agarose gel.
3. Gently pour the agarose solution which was warm into the gel platform.
4. Until the agarose gets solidified, the gel was not disturbed.
5. The submarine gel tank was filled with 1XTAE buffer.

6. The gel platform was kept inside tank with caution and its buffer level was not allowed to exceed 0.5cm above the gel.

7. With10µl HELINI 100bp DNA Ladder, PCR Samples and gel loading dye were added.

8. Until the dye reaches 3/4th distance of the gel. At 50Velectrophoresis was done.

9. The Gel was observed in Ultraviolet Transilluminator and the pattern of bands was detected.

TESTS TO DETERMINE METALLO BETA LACTAMASES AMONG NON FERMENTERS

SCREENING FOR MBLS

Imipenem: 10µg (IPM) and Ceftazidime: 30 µg (CAZ) resistant Pseudomonas aeruginosa isolates was taken for screening MBL production. Antibiotic sensitivity was done by the Kirby-Bauer disc diffusion method as recommended by CLSI.

CONTROLS

Pseudomonas aeruginosa (blaVIM and blaIMP positive) – positive control.

Pseudomonas aeruginosa ATCC 27853 - Negative control.

CONFIRMATION OF MBL PRODUCTION

All isolates positive for MBL production by screening test were subjected to:

COMBINED DISC TEST/DISC POTENTIATION TEST[45]

A lawn culture of 0.5 McFarland equivalent suspension of test organism was done in Mueller Hinton Agar plate. Two disc of Ceftazidime 30µg/Imipenem 10µg were placed in the plate with centre to centre at a distance of 25mm. 5 µl of 0.5 M EDTA (930 mg) was pipetted on one disk of Imipenem and Ceftazidime. After incubation at 37°C for 18-20 hours, increased zone of inhibition by 7 mm or more around any or all of the two discs with EDTA or increase in 5-28 mm inhibition around only CAZ-

EDTA disc when compared to Imipenem, Ceftazidime discs alone, respectively, were considered to be MBL producers.

METHODS TO DETECT AMP C PRODUCTION

SCREENING OF AMP C PRODUCTION

All isolates were screened for cefoxitin susceptibility and those which had a zone diameter of ≤ 18 mm were suspected to be AmpC producers.

AMPC DISK TEST[52]

In a MHA plate, a lawn culture of *E. coli* ATCC 25922 was made. Sterile saline (20µl) was put on the sterile disks (6 mm) which was later inoculated with several colonies of test organism. Cefoxitin disk (almost touching) was placed on a fresh inoculated plate. The inoculated disk was then placed adjacent to cefoxitin disk and incubated overnight at 35°C. The inference was made as follows:

Flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk is produced was taken as positive. A negative test had an undistorted zone.

CEFTAZIDIME IMIPENEM ANTAGONISM TEST (CIAT)[51, 53]

AmpC production was also confirmed by Ceftazidime - imipenem antagonism test (CIAT) (for inducible/chromosomal *AmpC* detection). In the disk antagonism tests, the test strain was exposed to disks of ceftazidime-imipenem which were placed at a distance. Flattening of the radius of the zone of inhibition produced on the side nearest to the imipenem disk indicated inducible AmpC production.

TESTS TO DETECT METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

CEFOXITIN DISC DIFFUSION TEST[60]

The test was performed by placing 30µg of cefoxitin disc in the Mueller Hinton Agar plate without NaCl supplementation inoculated with test organism. The plate was kept in incubator at a temperature of 37˚c. The zone of inhibition was determined after 24 hrs and the zone size was interpreted as

Susceptible: ≥21mm

Resistant : ≤ 21 mm

STATISTICAL ANALYSIS

The test outcome was observed, recorded and analysed. The data that were analysed was presented in the form of statistical tables, pie charts and histograms if necessary in appropriate places. P values were calculated by Chi –Square test. The data were documented and studied in detail. The documented data was further discussed in detail and compared with other similar studies published in reputed scientific journals.

RESULTS

Patients with Chronic Suppurative Otitis Media(CSOM) attending ENT OP at Government Kilpauk Medical College and Hospital, Chennai were studied for Extended Spectrum Beta Lactamases(ESBL), Metallo beta lactamases(MBL), AmpC beta lactamases and Methicillin Resistant Staphylococcus aureus(MRSA) . The study was done between January 2013 to August 2014. 212 patients with CSOM were studied and 173 bacterial isolates were isolated, identified and analysed for their antibiotic sensitivity pattern. Resistant isolates were identified and studied for Extended Spectrum Beta Lactamases(ESBL), Metallo beta lactamases (MBL), AmpC beta lactamases and Methicillin Resistant Staphylococcus aureus (MRSA) by various methods.

The observations were recorded and analysed. The results were as follows:

TABLE NO: 1 – AGE DISTRIBUTION (n=212).

AGE(YEARS)	MALE	FEMALE	PERCENTAGE
0-10	10	4	14(6.60%)
11-20	19	14	33(15.57%)
21-30	25	30	55(25.94%)
31-40	18	31	49(23.11%)
41-50	15	12	27(12.73%)
51-60	13	8	21(9.91%)
61-70	8	3	11(5.18%)
71-80	2	0	2(0.94%)
Total	110	102	**212**

Males and females within the age of 21- 30 years suffered from Chronic Suppurative Otitis Media when compared to other age groups. In our study, there was no statistical significance (p=0.557) in the age group causing CSOM.CSOM was equally distributed among males and females of all age groups.

TABLE NO: 2 – GENDER DISTRIBUTION (n=212)

GENDER	PERCENTAGE
Males	110(51.88%)
Females	102(48.11%)

Percentage of males with CSOM was 52% whereas the percentage of females was 48%.

CHART I

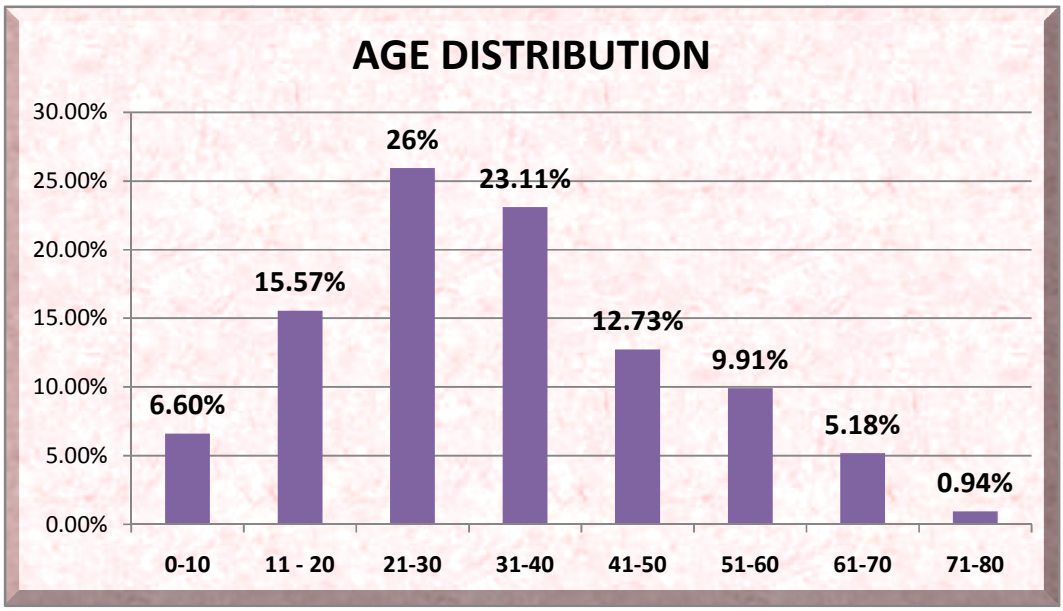


CHART II

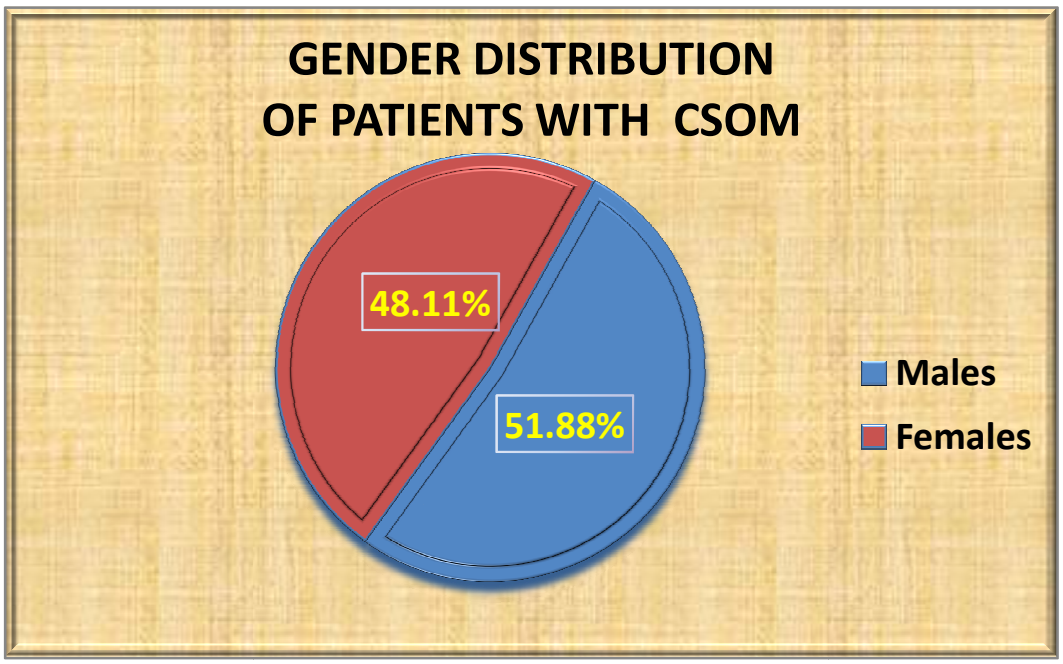


TABLE NO: 3 – RISK FACTORS OF PATIENTS (n=212)

Risk Factors	%
Annual Income of self/guardian	
≥ 100000	17(8.01%)
100000 -50000	71(33.49%)
≤ 50000	124(58.49%)
Education of self/parents	
Illiterate	60(28.30%)
SSC & below	103(48.59%)
HSC & Above	49(23.11%)
Habit of ear cleaning	
no habit	154(72.64%)
cleans ear	58(27.35%)

Out of 212 patients, 17(8.01%) had the annual income of ≥10000 whereas only 124(58.49%) had annual income of less than 50000. About 60(28.30%) were illiterates and 154(72.64%) did not have regular ear cleaning habits**.**

TABLE NO: 4 - SYMPTOMS OF CSOM (n=212)

Symptoms	**%**
Discharge only	112 (52.83%)
Discharge with deafness	51(24.06%)
Discharge with earache	28(13.21%)
Discharge ,earache and deafness	21(9.90%)
Total	**212**

Out of 212 patients with CSOM, 112(52.83%) had the presenting complaint of only discharge in the ear whereas 21(9.90%) presented with discharge, earache and deafness**.**

TABLE NO: 5 – SMEAR vs. CULTURE (n=212)

	Direct Smear Positive	**Direct Smear Negative**
Culture Positive	153(72.17%)	4(1.89%)
Culture Negative	0	55(25.94%)

153(72.17%) patients showed both direct smear and culture positive.4 (1.89%) were direct smear negative and culture positive.

55(25.94%) were both direct smear and culture negative. None of them were direct smear positive and culture negative.

TABLE NO: 6 - CULTURE POSITIVITY (n=212)

Culture Positive	157(74.06%)
Culture Negative	55(25.94%)
Total	**212**

Number of swabs that were culture positive was 157(74.06%) and number of sterile pus swabs was 55(25.94%).

TABLE NO: 7 - PURE vs. MIXED GROWTH (n=157)

ORGANISM	**PERCENTAGE**
Monomicrobial	141(89.80%)
Polymicrobial	16(10.19%)

Majority of cases 141(89.80%) showed monomicrobial infection and 16(10.19%) showed polymicrobial infection.

CHART III

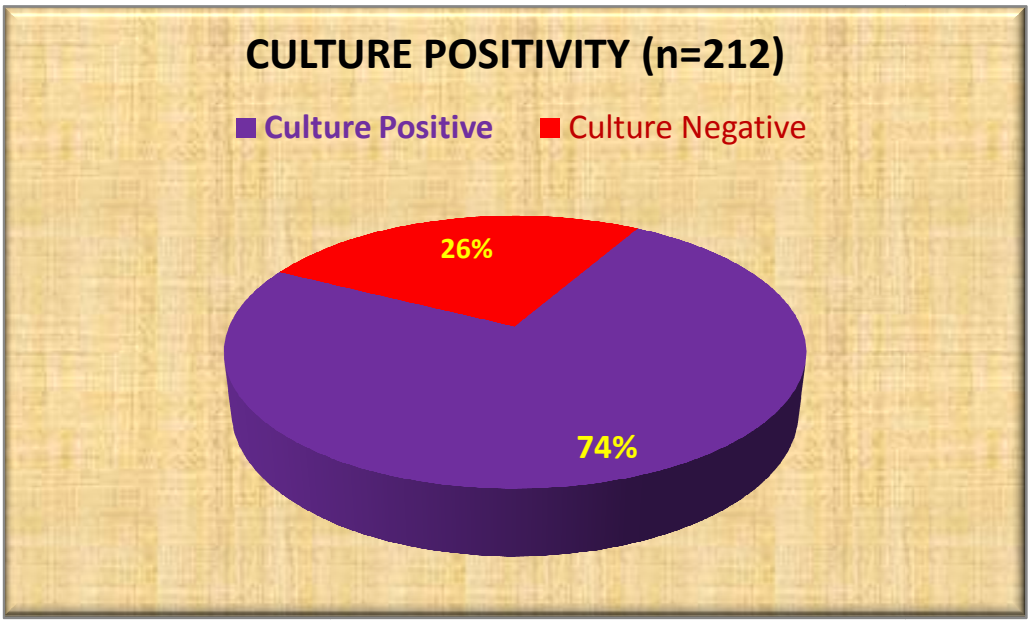


CHART IV

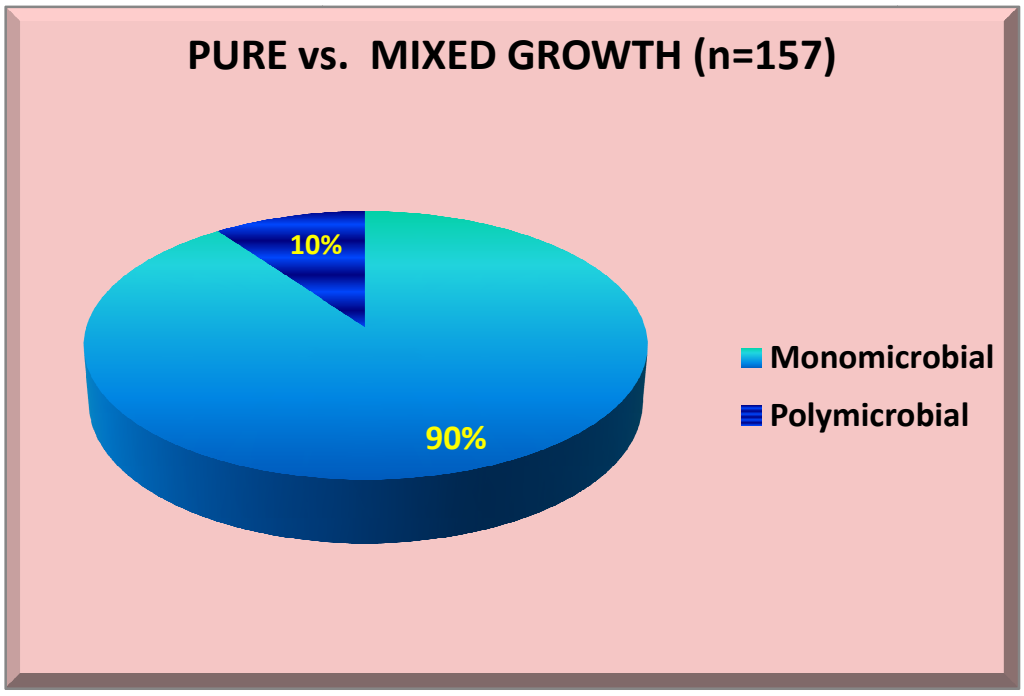


TABLE NO: 8 - GRAM POSITIVE Vs. GRAM NEGATIVE (n=173)

ORGANISM	PERCENTAGE
Gram negative bacilli	113(65.32%)
Gram positive cocci	60(34.68%)
Total	**173**

Out of 173 isolates from 212 patients, total number of Gram Positive cocci was 113(65.32%) and total number of Gram Negative bacilli was 60(34.68%)

TABLE NO: 9- DISTRIBUTION OF ORGANISMS (n=173)

ORGANISMS	%
Pseudomonas aeruginosa	80(46.24%)
Staphylococcus aureus	46(26.59%)
Klebsiella pneumoniae	25(14.45%)
Coagulase Negative Staphylococcus aureus	9(5.20%)
Proteus mirabilis	7(4.05%)
Enterococcus faecalis	5(2.89%)
Escherichia coli	1(00.58%)
Total	**173**

Out of 173 isolates, isolated from 212 patients with CSOM, Pseudomonas aeruginosa 80(46.24%) was the most common organism isolated in Gram Negative bacilli followed by Klebsiella pneumoniae 25(14.45%). Staphylococcus aureus 46(26.59%) was the common organism isolated among the Gram Positive cocci.

CHART V

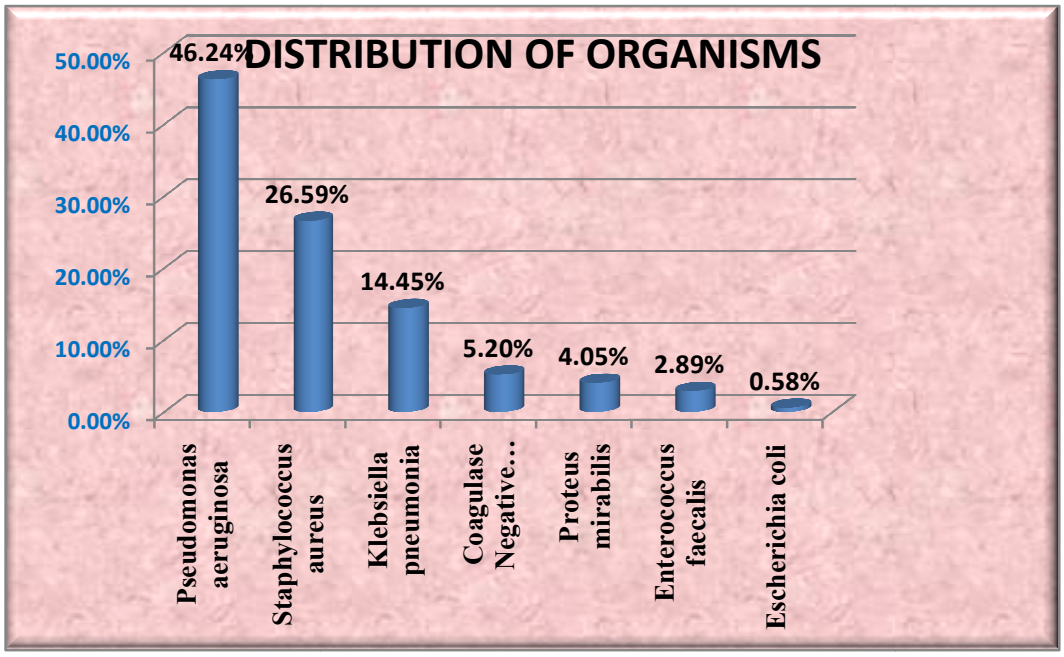


CHART VI

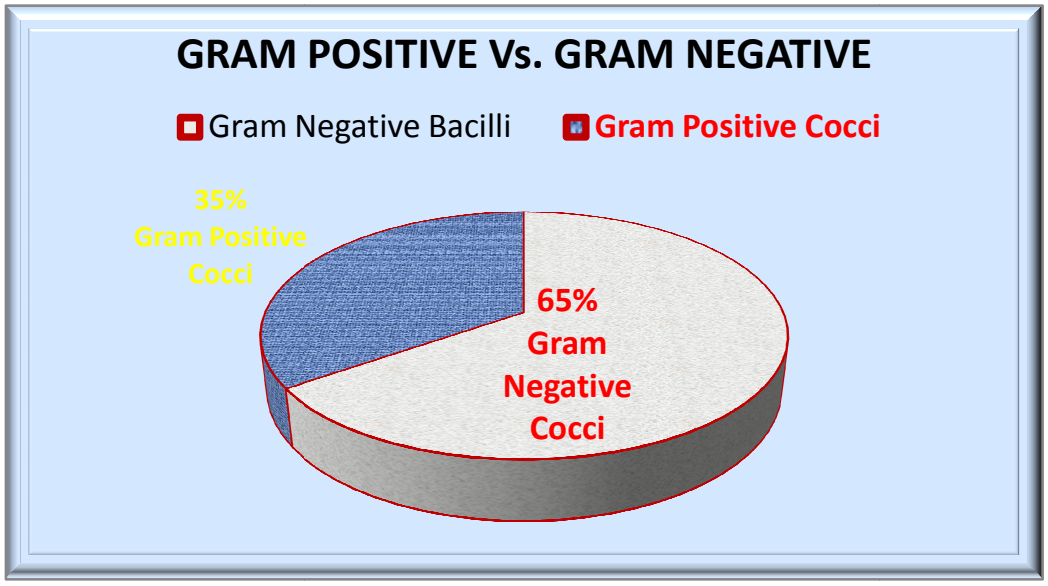


TABLE NO: 10 – ANTIBIOTIC SENSITIVITY OF GRAM NEGATIVE ISOLATES (n=113)

Antibiotics	Pseudomonas aeruginosa (n=80)	Klebsiella pneumonia (n=25)	Proteus mirabilis(n=7)
Amoxycillin	Not Tested	7(28.00%)	2(28.57%)
Amoxycillin - clavulinic acid	63(78.75%)	17(68%)	6(85.71%)
Cefoxitin	72(90%)	21(84%)	7(100%)
Cephalexin	52(65%)	13(52%)	4(57.14%)
Cefotaxime	74(92.50%)	15(60%)	7(100%)
Ceftazidime	74(92.50%)	15(60%)	7(100%)
Amikacin	77(96.25%)	20(80%)	6(85.71%)
Gentamicin	59(73.75%)	14(56%)	3(42.85%)
Ciprofloxacin	58(72.50%)	12(48%)	3(42.85%)
Ofloxacin	76(95%)	18(72%)	6(85.71%)
Piperacillin – Tazobactam	78(97.50%)	25(100%)	7(100%)
Imipenem	80(100%)	25(100%)	7(100%)

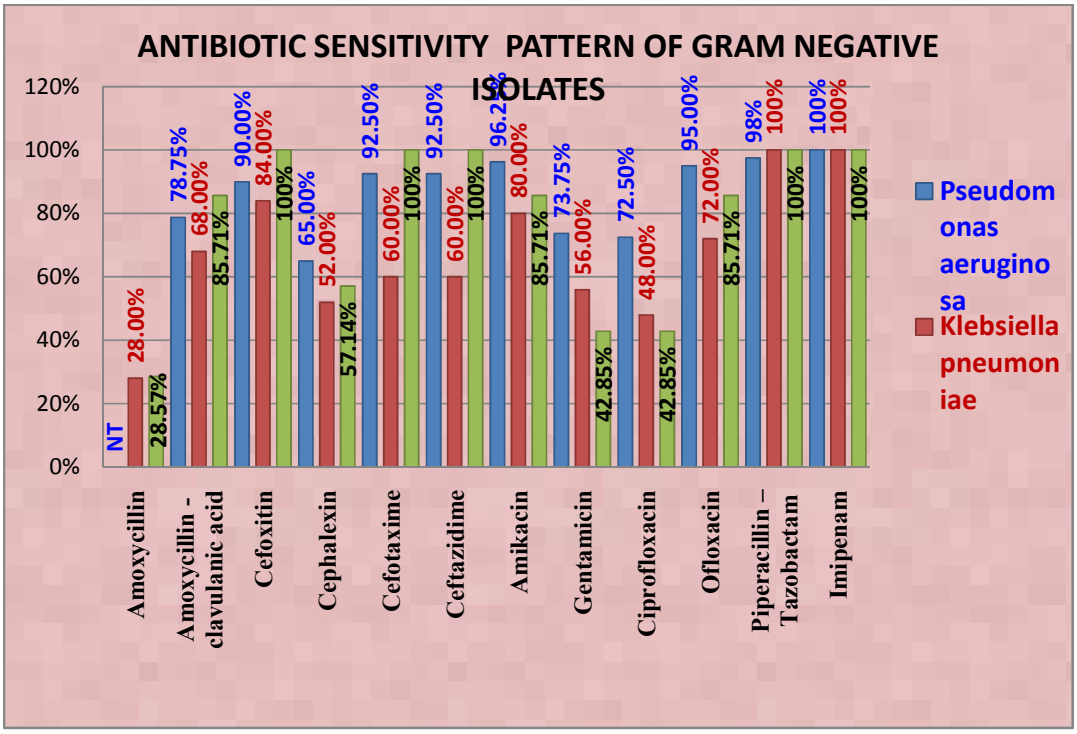
All gram Negative bacilli isolates showed 100% sensitivity to imipenem. Klebsiella pneumoniae and Proteus mirabilis showed 100%

sensitivity to Piperacillin/Tazobactam but Pseudomonas aeruginosa showed 78(97.50%) sensitivity to Piperacillin/Tazobactam.

Pseudomonas aeruginosa showed 77(96.25%) and 76(95%) sensitivity to amikacin and ofloxacin respectively where as Proteus mirabilis showed 6(85.71%) sensitivity to amikacin.

Amoxycillin showed less than 30% sensitivity to Klebsiella pneumoniae and Proteus mirabilis.

CHART VII



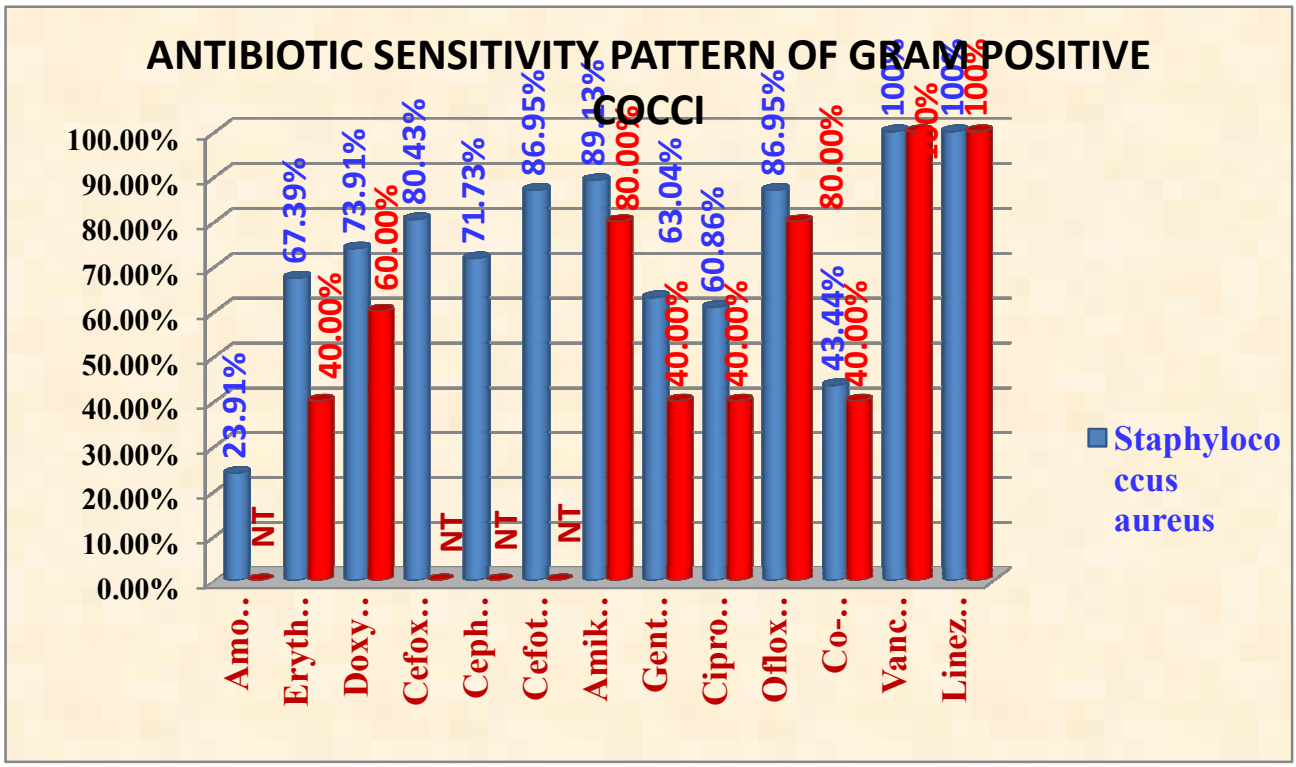
NT - Not Tested

TABLE NO: 11 – ANTIBIOTIC SENSITIVITY OF GRAM POSITIVE ISOLATES (n=60).

Antibiotics	Staphylococcus aureus(n=46)	Coagulase Negative Staphylococcus aureus(n=9)	Enterococcus faecalis(n=5)
Amoxycillin	11(23.91%)	3(33.33%)	Not Tested
Erythromycin	31(67.39%)	5(55.55%)	2(40%)
Doxycycline	34(73.91%)	7(77.77%)	3(60%)
Cephalexin	33(71.73%)	6(66.66%)	Not Tested
Cefotaxime	40(86.95%)	8(88.88%)	Not Tested
Amikacin	41(89.13%)	8(88.88%)	4(80%)
Gentamicin	29(63.04%)	6(66.66%)	2(40%)
Ciprofloxacin	28(60.86%)	5(55.55%)	2(40%)
Ofloxacin	40(86.95%)	8(88.88%)	4(80%)
Co-trimaxazole	20(43.44%)	4(44.44%)	2(40%)
Vancomycin	46(100%)	9(100%)	5(100%)
Linezolid	46(100%)	9(100%)	5(100%)
Cefoxitin	37(80.43%)	7(77.77%)	Not Tested

All the Gram Positive cocci were 100% sensitive to Vancomycin and Linezolid. Staphylococcus aureus and Coagulase Negative Staphylococcus aureus showed 89.13% and 86.95% sensitivity to amikacin to Ofloxacin

CHART VIII



NT - Not Tested

respectively. Staphylococcus aureus showed sensitivity of 86.95% and 71.73% to cefotaxime and cephalexin respectively.

Out of 5 Enterococci faecalis, 80% were sensitive to both Ofloxacin and amikacin.

TABLE NO: 12– SCREENING TEST FOR ESBL

Gram Negative isolates	Resistant to ceftazidime & cefotaxime
Klebsiella pneumoniae (n=25)	10(40%)
Pseudomonas aeruginosa (n=80)	6(7.5%)

Out of 113 isolates, 10 Klebsiella pneumoniae and 6 Pseudomonas aeruginosa were resistant to both drugs.

All the 10 isolates were subjected to phenotypic confirmatory test /Disc Potentiation test and MIC reduction test to detect the presence of ESBL Production .The 6 resistant Pseudomonas aeruginosa isolates were subjected to Disc Potentiation test with EDTA test for MBL detection.

TABLE NO: 13 – ESBL PRODUCERS BY PHENOTYPIC CONFIRMATORY TEST (n=33)

Enterobacteriaceae	Phenotypic Confirmatory Test – Positive
33	10(30.30%)

By the phenotypic confirmatory method, all 10 Klebsiella pneumoniae isolates were identified as ESBL producers

TABLE NO 14–MINIMUM INHIBITORY CONCENTRATION OF ISOLATES TOCEFTAZIDIME (µg/ml) (n=10)

Isolate	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048
Klebsiella pneumoniae	0	0	0	0	3	2	2	0	0	0	2	1	0

Minimum inhibitory concentration of isolates to Ceftazidime for the ESBL producing organism in the study was between 8(µg/ml) of agar to 1024(µg/ml) of agar.

TABLE NO: 15- MIC ISOLATES TO CEFTAZIDIME WITH CLAVULINIC ACID (n=10)

Isolate	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048
Klebsiella pneumoniae	3	2	2	0	0	0	2	0	1	0	0	0	0

Minimum inhibitory concentration of isolates to Ceftazidime for the ESBL producing organism in the study was between 0.5(µg/ml) of agar to 128(µg/ml) of agar in presence of 4µg/ml clavulanic acid in the agar showing reduction in ≥3 doubling dilution.

TABLE NO - 16: SCREENING OF MBL (n= 80) PSEUDOMONAS AERUGINOSA

Antibiotics	Sensitive	Resistant
Ceftazidime	74(92.5%)	6(7.5%)
Imipenem	80(100%)	0

All the Pseudomonas aeruginosa isolates resistant to Ceftazidime and imipenem were screened as probable MBL producers. Thus out of 80 isolates, 6 isolates were resistant to Ceftazidime whereas all the Pseudomonas aeruginosa isolates showed 100% sensitivity to imipenem.

TABLE NO – 17: MBL Detection (n=6) - Disc Potentiation test with EDTA

Ceftazidime – EDTA Test		
No of Isolates	**Positive**	**Negative**
6	**1(16.66%)**	**5(83.33%)**

By Ceftazidime – EDTA Test method, out of 6 isolates 1 (16.66%) was found to be MBL producer. Whereas by imipenem –EDTA method none of them were MBL producing.

TABLE NO: 18-- AMP C PRODUCERS AMONG GRAM NEGATIVE BACILLI (n=113)

Gram Negative Bacilli	**%**
Enterobacteriaceae	3(2.65%)
Pseudomonas aeruginosa	6(5.30%)
Total	9(7.96%)

By AmpC disk test and ceftazidime -imipenem antagonism test, 9(7.96%) were found to be Amp C producers

TABLE NO: 19 GENE IDENTIFICATION IN ESBL POSITIVE ISOLATES OF TEM, SHV and CTX-M.

Organism	No. tested	TEM Positive	SHV Positive	CTX-M Positive
Klebsiella Pneumoniae	10	3(30%)	4(40%)	3(30%)

All the Klebsiella pneumoniae isolates that were confirmed as ESBL producers by phenotypic confirmatory test were subjected to PCR for TEM, SHV and CTX-M gene identification. Out of 10 isolates subjected to PCR, 3(30%) were positive for TEM gene, 4(40%) and 3(30%) were positive for SHV and CTX-M genes respectively.

TABLE NO: 20 - MRSA DETECTION IN STAPHYLOCOCCUS AUREUS BY CEFOXITIN DISC DIFFUSION METHOD (n=46)

Staphylococcus aureus	%
MSSA ≥ 21mm	37(80.43%)
MRSA ≤ 21mm	9(19.56%)
Total	**46**

Out of 46 Staphylococcus aureus, 9(19.56%) were MRSA positive by cefoxitin disc diffusion method.

TABLE NO: 20 a – DETECTION OF METHICILLIN RESISTANT IN CONS (n=9)

CONS – MR	**%**
MS – CONS ≥ 25mm	7(77.77%)
MR – CONS ≤ 24mm	2(22.22%)
Total	9

By cefoxitin disc diffusion method, out of 9 Coagulase Negative Staphylococcus aureus isolated, 2(22.22%) were Methicillin Resistant CONS.

CHART IX

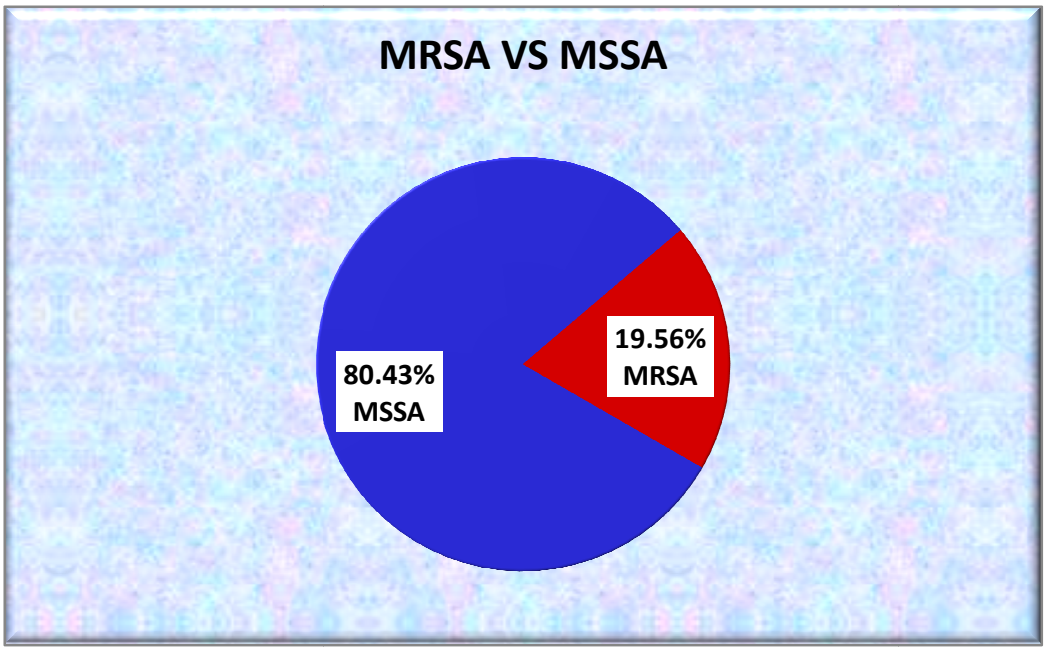


TABLE NO -21: OUTCOME OF THE STUDY (n=189) THE REMAINING 23 LOST FOLLOW UP

Outcome	No (%)	MRSA	ESBL	MBL	Non ESBL/MBL/AmpC/MBL
Recovered	169 (89.41%)	9 (5.32%)	7 (4.14%)	0	153 (90.53%)
Operated	20 (10.5%)	0	3 (15%)	1 (5%)	16 (80%)

The outcome was noted in 189 cases only and the remaining lost follow up .out of 189 cases recovery was seen in 169(89.41%) and 9(5.32%) were MRSA Positive, 7(4.14%) were ESBL positive and the remaining 153(90.53%) did not harbor any ESBL/MBL/AmpC/MRSA producers.

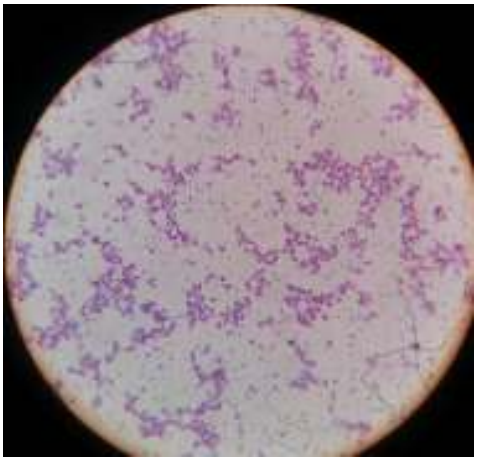
20(10.5%) of them were operated for CSOM, out of them 3(15%) were ESBL positive and 1(5%) was MBL positive. 16(80%) of them were non ESBL/MBL/AmpC/MRSA producers.

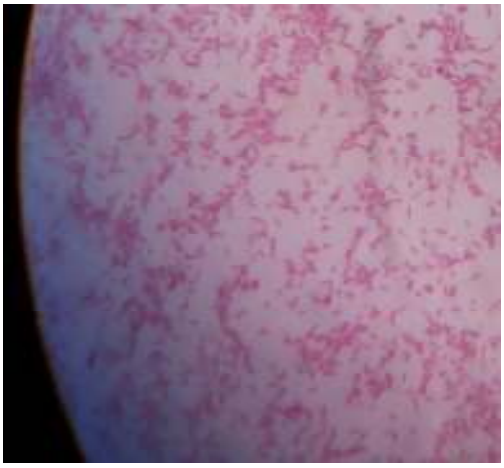
STERILE SWAB

CSOM

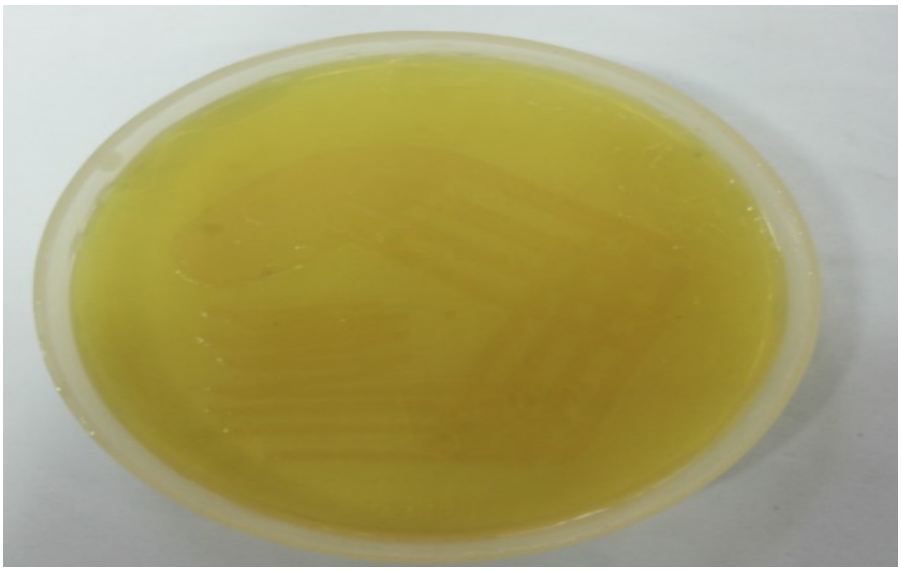
GRAM POSITIVE COCCI IN CLUSTERS

GRAM NEGATIVE BACILLI

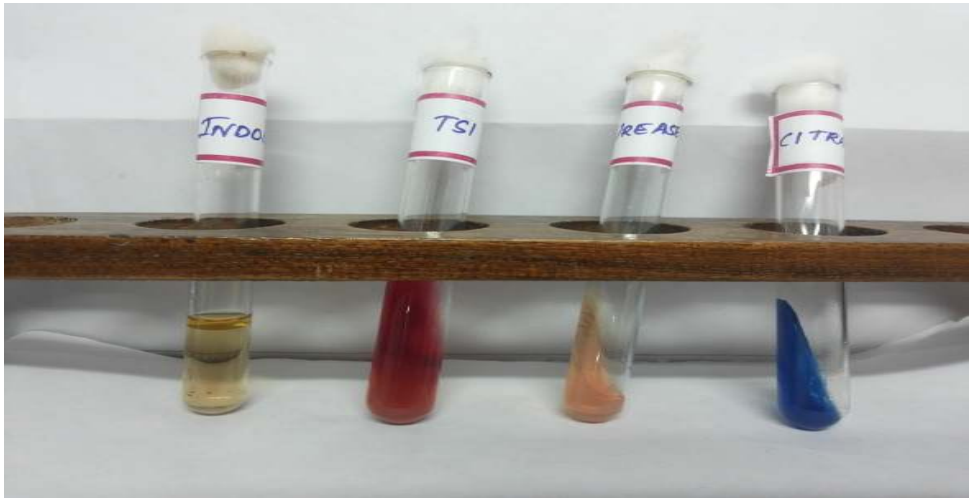




PSEUDOMONAS IN NA



BIOCHEMICAL REACTONS OF PSEUDOMONA AERUGINOSA

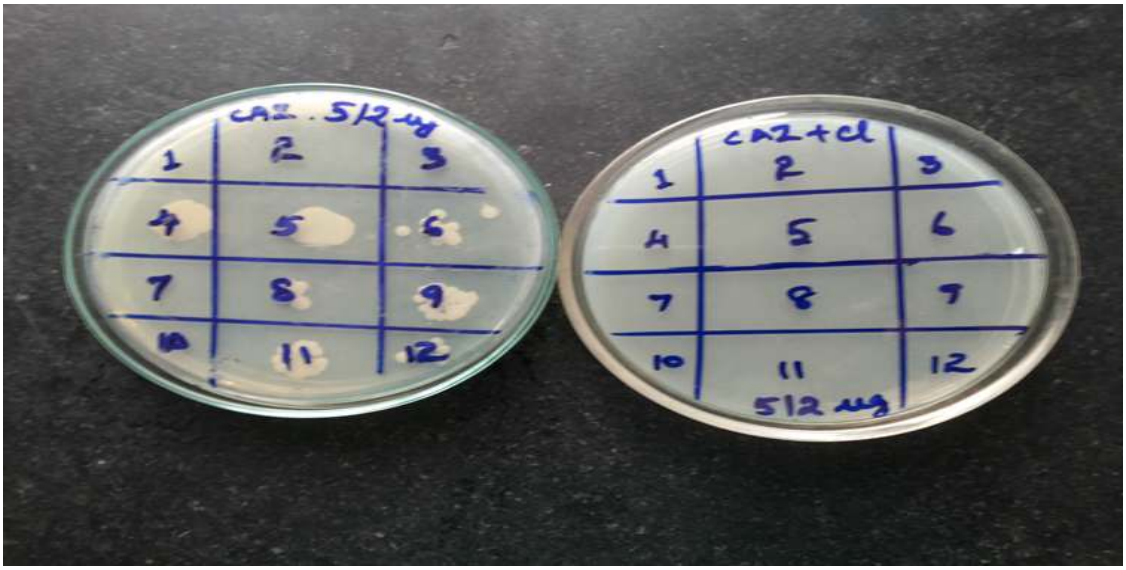


PHENOTYPIC CONFIRMATORY FOR ESBL

CAZ=ceftazidime 30 µg

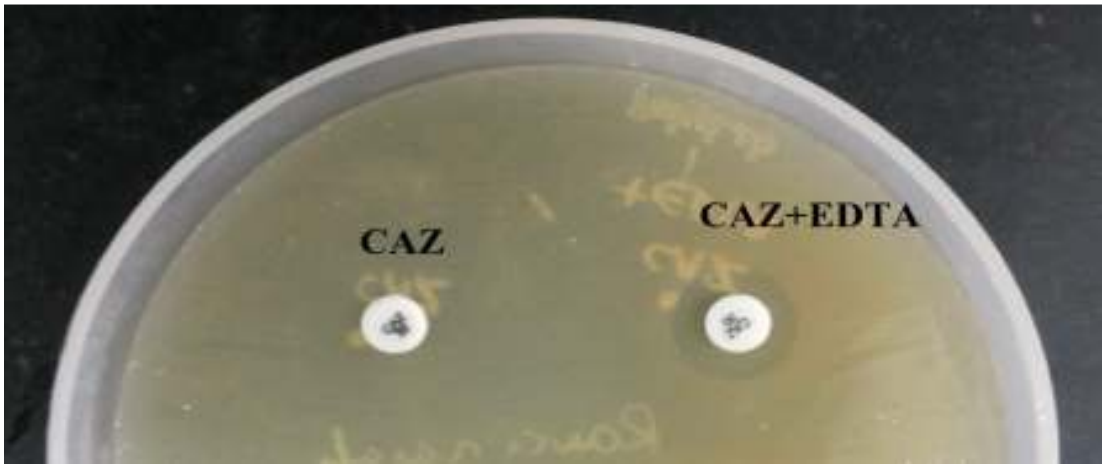
CAZ+CLAV=ceftazidime 30 µg+clavulanic acid 10 µg (≥5mm than CAZ)

MIC REDUCTION TEST



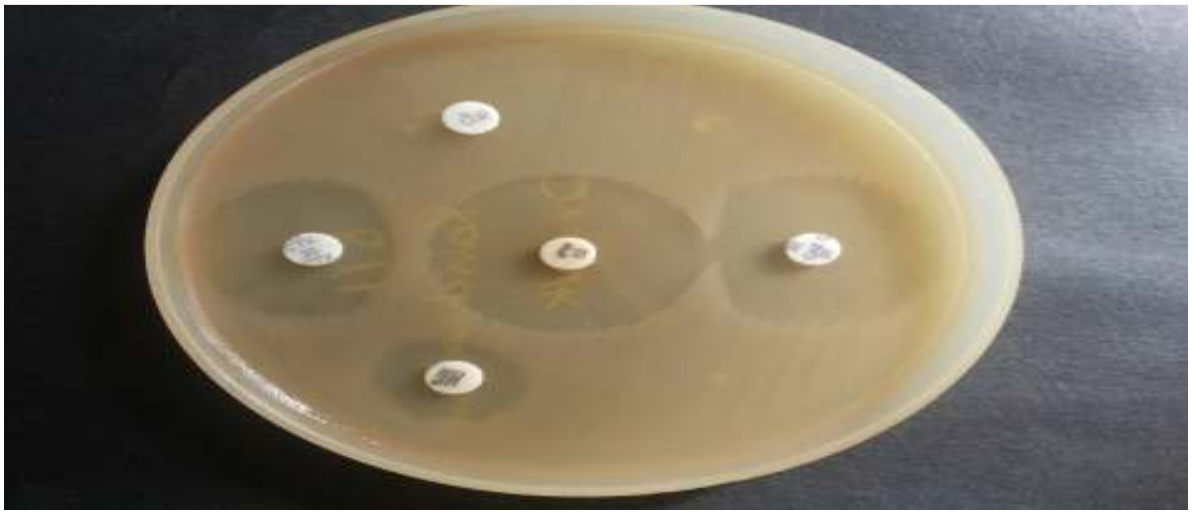
MIC to 512 µg of ceftazidime along with clavulanic acid

MBL DETECTION



Combined disc test Ceftazidime+EDTA

CEFTAZIDIME – IMIPENAM ANTAGONISM TEST



Flattening of the radius of the zone of inhibition produced

on the side nearest to the imipenem disk

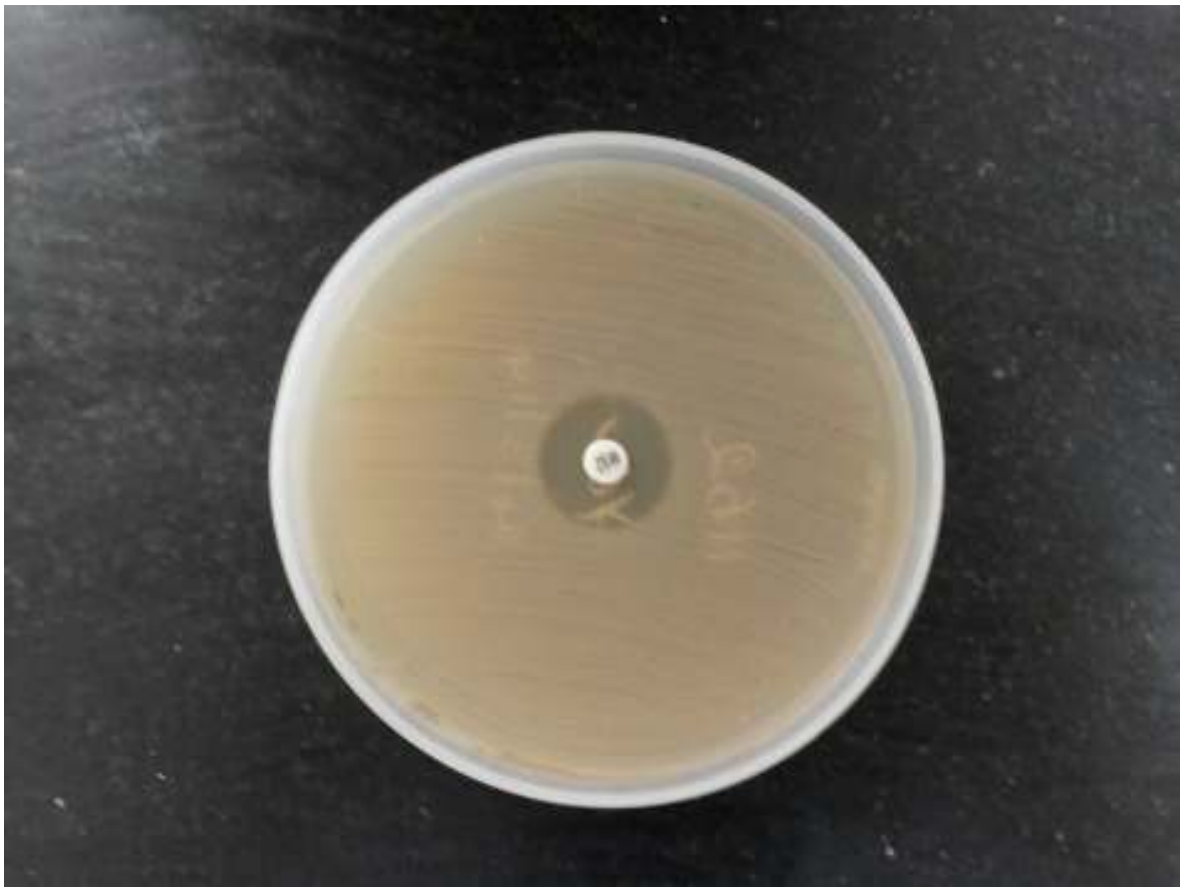
CEFTAZIDIME – IMIPENAM ANTAGONISM TEST

Flattening of the radius of the zone of inhibition produced on the side nearest to the imipenem disk

AMP –C DISK TEST

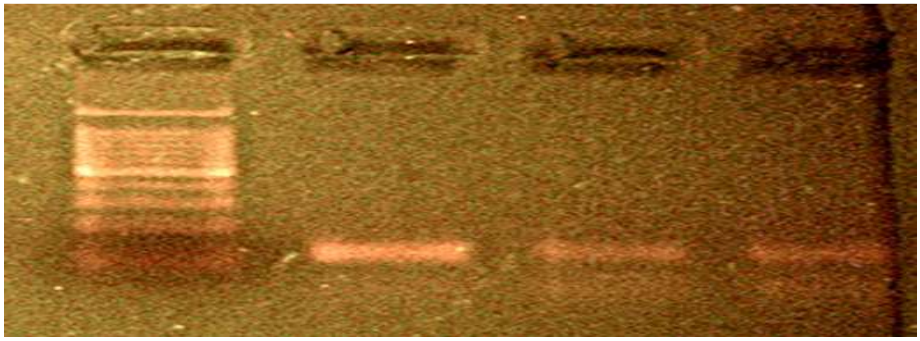
Flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk

DETECTION OF MRSA USING CEFOXITIN DISC



Cefoxtin Zone Size = ≤21mm - MRSA

CTX-M

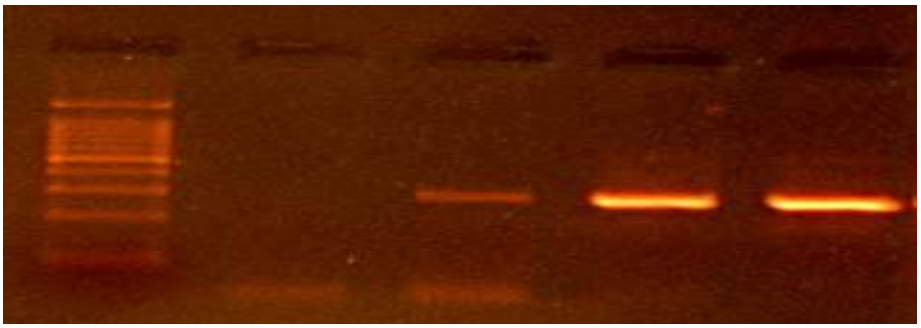


Lane One : DNA Ladder

Lane Two : 120 bp

Lane Three : 120 bp

SHV

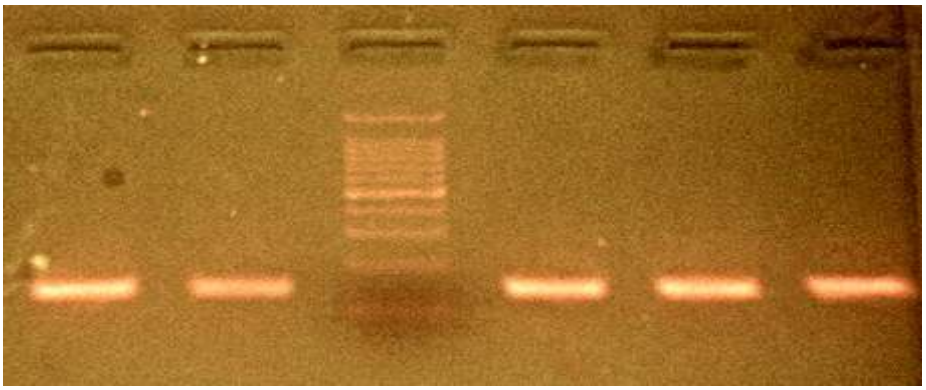


Lane One : DNA Ladder

Lane Two : Negative

Lane Three : 276 bp

TEM



Lane One	:	DNA Ladder
Lane Two	:	148 bp
Lane Three	:	DNA Ladder
Lane Four	:	148 bp
Lane Five	:	148 bp

DISCUSSION

This study aims at determining the common bacterial isolates, their antimicrobial susceptibility pattern, MRSA and ESBL and Amp C producers causing Chronic Suppurative Otitis Media. This study was conducted in all age groups among 212 patients who were diagnosed as CSOM. Both male and female patients were observed. The observations were recorded, analyzed and discussed as follows.

As per table 1 all age groups were included. Age of patients studied ranges from 0-80years, the oldest case was 76 years of age. Patients within the age of 21-30 years were 55(25.94%) and in the age group of 31-40 years were 47(22.16%). which is similar to the observations in the study done by Shashidhar Viswanath et al[21]and Rejitha IM[72] et al which showed 21(22.34%) and 30% in the age group of 21-30 years. While studies done by Oguntibeju OO et al[69] showed that CSOM was common below 30 years of age.

As per table 2 In our study, Males 110(51.88%) and Females 102(48.11%) were suffering from CSOM which shows male preponderance. These findings are in accordance with the observations by Harvinder Kumar et al[3] and Poorvey et al[4] whereas Maji et al[5] and Rajat

Prakash et al[6] shows females are affected more than males. According to our study, males and females in the age group of 21-30 years suffered from Chronic Suppurative Otitis Media more significantly than other age groups.

As per table 3 Risk factors of CSOM such as illiteracy, low annual income and ear cleaning habits were assessed. 124(58.64%) patients had annual income of patients below 50000.103(48.59%) patients had studied 10^{th}std or below. 154(72.64%) patients had no habit of cleaning the ear regularly. This is in accordance with Md Mazharul Shaheen et al[14].

As per table 4 Discharge alone was seen in 115(52.83%). Deafness was associated with discharge in 51(24.06%). Earache was associated with discharge in 28(13.21%). All the three symptoms – discharge, deafness and earache was found in 21(9.09%). This is similar to the findings observed by Shashidhar Viswanath et al[21] and Karan Sharma et al[8].

As per table 5 Direct smear positive and culture positive was seen in 153(75%). In a study done by Shyamala R et al[73] 93% were found to be both smear as well as culture positive.

As per table 6, 147(89.37%) were monomicrobial and 17(10.69%) showed polymicrobial pattern. This finding corroborates well with the

findings reported by Karan Sharma et al[8], Poorvey et al[4], Sanjay Kumar et al[20] and Rajat Prakash et al[6].

As per table 7 Number of culture positive ear swabs were 157(74.06%) and 55(25.94%) were culture negative. This was coinciding with the studies done by Oguntibeju OO et al[69].This was contrast to the study done by Sateesh Kumar Malkappa et al[**74**] and Harvinder Kumar et al[3]. This could be due to prior antibiotic therapy and also due to microbial resistance to the antibiotics leading onto continuous purulent discharge from the ear.

As per table 8: In our study, Gram negative bacilli were predominant in 113(65.69%) cases of CSOM and Gram Positive cocci was found in 57(34.30%) which is comparable with the study done by Harvinder Kumar et al[3] which showed that 59.94% gram negative bacilli and 37.7% were Gram Positive cocci. Similarly, Sateesh Kumar Malkappa et al[74] where gram negative bacilli were 69.84% and gram positive isolates were 31%. A contrast study with the predominance of gram positive cocci was by various investigators Poorvey et al[4] and Rajat Prakash et al[6].

As per table 9 in the present study, the predominant organism isolated was Pseudomonas aeruginosa 80(46.10%) followed by Staphylococcus aureus 46(26.59%), Klebsiella pneumoniae 25(14.45%), Coagulase negative Staphylococcus aureus 9(5.20%), Proteus mirabilis 7(4.05%), Enterococcus faecalis 5(2.89%) and Escherichia coli 1(00.58%). This is in accordance to the fact reported by Harvinder Kumar et al[3], Oguntibeju OO et al[69], Shashidhar Viswanath et al[21], Maji et al[5], Poorvey et al[4], Sateesh Kumar Malkappa et al[74], and Sanjay Kumar et al[20] that Pseudomonas aeruginosa was the most common organism in CSOM.

Arti Agrawal et al[75] found that Staphylococcus aureus was the predominant organism in their study, Rajat Prakash et al[6] also observed that Staphylococcus aureus was the commonest organism causing CSOM followed by Pseudomonas aeruginosa whereas Saini et al[76] reported that in Pediatric age group, Staphylococcus aureus was the commonest organism and in adult, Pseudomonas aeruginosa was the predominant organism in CSOM

As per table 10in the present study among the Gram negative isolates, Pseudomonas aeruginosa showed 77(96.25%) sensitivity to amikacin and 77(95%) were sensitivity to ofloxacin. All Gram negative isolates were 100% sensitive to imipenem and Piperacillin –Tazobactam

except Pseudomonas aeruginosa which showed 78(97.50%) sensitivity to Piperacillin –Tazobactam.

In the study done by Poorvey et al[4] 90-100% sensitivity was reported among Gram negative isolates to amikacin. In our study, among Klebsiella pneumoniae isolates, 25(100%) sensitivity was reported for imipenem and Piperacillin/Tazobactam. 20 (80%) and 18(72%) were sensitive to amikacin and ofloxacin respectively whereas it showed 14(56%) sensitivity to gentamicin. 15(60%) sensitivity was found to ceftazidime and cefotaxime. Among Proteus mirabilis,6 (85.71%) were sensitive to amikacin and ofloxacin.

Amoxycillin showed less than 30% sensitivity to Klebsiella pneumoniae and Proteus mirabilis.

As per table 11 of the 46 Staphylococcus aureus, 41(89.13%) was sensitive to amikacin, 40(86.95%) to ofloxacin and 20(43.44%) to cotrimaxozole. Erythromycin showed 31(67.39%) sensitivity. 100%sensitivity was reported to vancomycin and linezolid. Enterococcus faecalis was 4(100%) sensitive to vancomycin and linezolid.

In a study done by Rajat Prakash et al[6] Staphylococcus aureus show 95% sensitivity to amikacin and 82%sensitivity to ciprofloxacin. Harvinder Kumar et al[3] also reported that Staphylococcus aureus show 90% sensitivity to amikacin and 68% sensitivity to erythromycin. Oguntibeju et al[69] reported that Staphylococcus aureus shows 40% sensitivity to cotrimaxazole.

As per table 12: ESBLs were screened by observing resistance to third generation cephalosporin - cefotaxime and ceftazidime in which out of 33 Gram negative bacilli 10(30.30%) were ESBL producers. The distribution of ESBL are as follows, out of 25 Klebsiella pneumoniae, 10(40%) were found resistant to both ceftazidime and cefotaxime. Proteus mirabilis and Escherichia coli showed 100% sensitivity to both cephalosporins.

As per table13: 10 Klebsiella pneumoniae which were resistant to 3rd generation cephalosporin were further confirmed by phenotypic confirmatory test/disc potentiation test. This method showed 10 ESBL producers.This is in accordance with the study done by IM Rejitha et al[72] and NB Swarooprani et al[77].

As per table 14 and 15: Minimum inhibitory concentration (MIC) of Ceftazidime with clavulanic acid for the ESBL producers was done by Agar dilution method for 10 isolates, MIC of the isolates ranged from 8µg/ml to 512µg/ml. in the presence of ceftazidime with 4µg/ml clavulinic acid, MIC of ESBL isolates ranged from 0.5µg/ml to 64µg/ml showing ≥3 doubling dilution reduction in MIC. These observations confirmed ESBL producers in the test isolates.

As per table 16 All the Ceftazidime and imipenem resistant Pseudomonas aeruginosa isolates were screened as probable MBL producers. Thus out of 80 isolates, 6 isolates were resistant to Ceftazidime whereas all the Pseudomonas aeruginosa isolates were 100% sensitive to imipenem.

As per table 17 By Ceftazidime – EDTA Test method, out of 6 isolates 1 (16.66%) was found to be MBL producer. This is similar to study by Agarwal G et al[78].

As per table 18 Screening for Amp C production was done by observing resistance to cefoxitin in which of the 113 enterobacteriaceae, 12 were resistant to cefoxitin, out of which 9(7.96%) were Amp C producers. Amp C production was done by Amp C disk test and

ceftazidime – imipenem test. 3(2.65%) were Klebsiella pneumoniae and 6(5.30%) were Pseudomonas aeruginosa. This is similar to study done by Singhal et al[52] which reported that AmpC producers were about 8% among Gram negative isolates.

As per table 19: 10 ESBL producing enterobacteriaceae which were phenotypically confirmed as ESBL producers were subjected to genotypic test by Polymerase Chain Reaction (PCR).three genes such as SHV, TEM and CTX-M associated with ESBL production was studied using the relevant primers for the corresponding genes. TEM genes were detected in 3(30%) and SHV were detected in 4(40%).CTX-M was detected in 3(30%).

As per table 20: out of 46 Staphylococcus aureus isolates, 9(19.53%) were resistant to cefoxitin. 9 were MRSA which was detected by cefoxitin disc diffusion method. This was similar to Arti Agrawal et al[75] which showed 20% MRSA producers. Prakash M et al[79] also reported that 18% were MRSA. Anupkumar Shetty et al[80] reported that 36% were MRSA producers and Swarooprani NB et al[77] showed 41.1% MRSA producers. A contrasting study by IM Rejitha et al[72] showed 83.3% MRSA. In the present study, all MRSA strains showed 100% sensitivity to vancomycin and linezolid.

As per table 21: The outcome was noted in 189 cases only and the remaining lost follow up. Out of 189 cases recovery was seen in 169 and 9 was MRSA Positive, 7 were ESBL positive and the remaining 153 did not harbour any ESBL/MBL/AmpC/MRSA producers.

20 of them were operated for CSOM, out of them 3 were ESBL positive and 1 was MBL positive 16 of them were non ESBL/MBL/AmpC/MRSA producers.

In this period of emerging drug resistance amongst bacteria, episodic monitoring of the microbiological profile of CSOM along with clinical association is indispensable as precise antibiotics for empirical therapy depends on the local antibiotic policy, followed by alteration in treatment based on the culture and antimicrobial susceptibility results. Before managing with antibiotics (either systemic or local) culture of ear discharge should be done in all CSOM patients.

SUMMARY

Two hundred and twelve patients with CSOM between Jan 2013 to August 2014 formed the study group. Specimens obtained from study group were cultured and bacterial isolates were identified by standard microbiological techniques. All age groups were involved .The oldest being 76 years. Males and females in the age group of 21-30 years suffered from Chronic Suppurative Otitis Media significantly more than other age group**.** In our study, the percentage of males 110 (51.88%) and the percentage of females was 102 (48.11%).

Monomicrobial infection was 141 (89.80%) and Polymicrobial infection was 16 (10.19%). Culture positive ear swabs were 157(74.06%) and culture negative ear swabs were 55 (25.94%). The current study showed that Gram negative bacilli were 60(34.68%), the predominant bacteria isolated in patients with CSOM. Among Gram negative bacilli, pseudomonas aeruginosa was found to be the most common bacterial pathogen isolated in our study and Staphylococcus aureus was the most common Gram positive organism isolated followed by Klebsiella pneumoniae.

In the present study, antibiotic sensitivity of all Enterobacteriaceae showed 100% sensitivity to imipenem and Piperacillin/Tazobactam except Pseudomonas aeruginosa which showed 78(97.50%) sensitivity to Piperacillin /Tazobactam. 77(96.25%) Pseudomonas aeruginosa, 20 (80%) Klebsiella pneumoniae and 6 (85.71%) Proteus mirabilis were sensitive to amikacin respectively. 76(95%) Pseudomonas aeruginosa, 18(72%) Klebsiella pneumoniae, 6(85.71%) Proteus mirabilis showed sensitivity to ofloxacin respectively. Amoxycillin showed less than 30% sensitivity in all Gram negative isolates.

Antibiotic sensitivity of Staphylococcus aureus showed 100% sensitivity to vancomycin and linezolid. Among 46 Staphylococcus aureus, 41(89.13%) was sensitive to amikacin and 40(86.95%) to ofloxacin. Erythromycin showed 31(67.39%) sensitivity. Enterococcus faecalis was 4(100%) sensitive to vancomycin and linezolid.

ESBLs were screened by detecting resistance to third generation cephalosporin - cefotaxime and ceftazidime in which out of 33 Gram negative bacilli 10(30.30%) were ESBL producers. The distribution of ESBL are as follows, out of 25 Klebsiella pneumoniae, 10(40%) were found resistant to both ceftazidime and cefotaxime. Proteus mirabilis and Escherichia coli showed 100% sensitivity to both cephalosporins.

Phenotypic confirmatory test/disc potentiation test was done to confirm ESBL production. 10 Klebsiella pneumoniae which were resistant to 3rd generation cephalosporin were further confirmed by this method which showed 10 ESBL producers. Minimum inhibitory concentration (MIC) of ceftazidime with clavulanic acid for the ESBL producers was done by agar dilution method for 10 isolates, MIC of the isolates ranged from 8µg/ml to 512µg/ml. in the presence of ceftazidime with 4µg/ml clavulinic acid, MIC of ESBL isolates ranged from 0.5µg/ml to 64µg/ml showing ≥3 doubling dilution reduction in MIC. These observations confirmed ESBL producers in the test isolates.

10 ESBL producing enterobacteriaceae which were phenotypically confirmed as ESBL producers were subjected to genotypic test by Polymerase Chain Reaction (PCR).three genes such as TEM, SHV and CTX-M associated with ESBL production was studied using the relevant primers for the corresponding genes. TEM genes were detected in 3(30%) and SHV were detected in 4(40%).CTX-M was detected in 3(30%).

All the Pseudomonas aeruginosa isolates resistant to ceftazidime and imipenem were screened as probable MBL producers. Thus out of 80 isolates, 6 isolates were resistant to Ceftazidime whereas all the Pseudomonas aeruginosa isolates were 100% sensitive to imipenem. By

Ceftazidime – EDTA Test method, out of 6 isolates 1 (16.66%) was found to be MBL producer. Whereas by imipenem –EDTA method none of them were MBL producers.

Screening for Amp C production was done by observing resistance to cefoxitin in which of the 113 enterobacteriaceae, 9(7.96%) were Amp C producers. Amp C production was done by AmpC disk test and ceftazidime – imipenem test. 3(2.65%) were Klebsiella pneumoniae and 6(5.30%) were Pseudomonas aeruginosa.

Among 46 Staphylococcus aureus isolates, 9(19.53%) were resistant to cefoxitin. 9 were found to be MRSA by cefoxitin disc diffusion method. In the present study, all MRSA strains showed 100% sensitivity to vancomycin and linezolid.

The outcome was noted in 189 cases only and the remaining lost follow up. Out of 189 cases recovery was seen in 169 and 9 was MRSA Positive, 7 were ESBL positive and the remaining 153 did not harbour any ESBL/MRSA producers.

20 of them were operated for CSOM, out of them 3 were ESBL positive and 1was MBL positive 16 of them were not ESBL/MRSA producers.

CONCLUSION

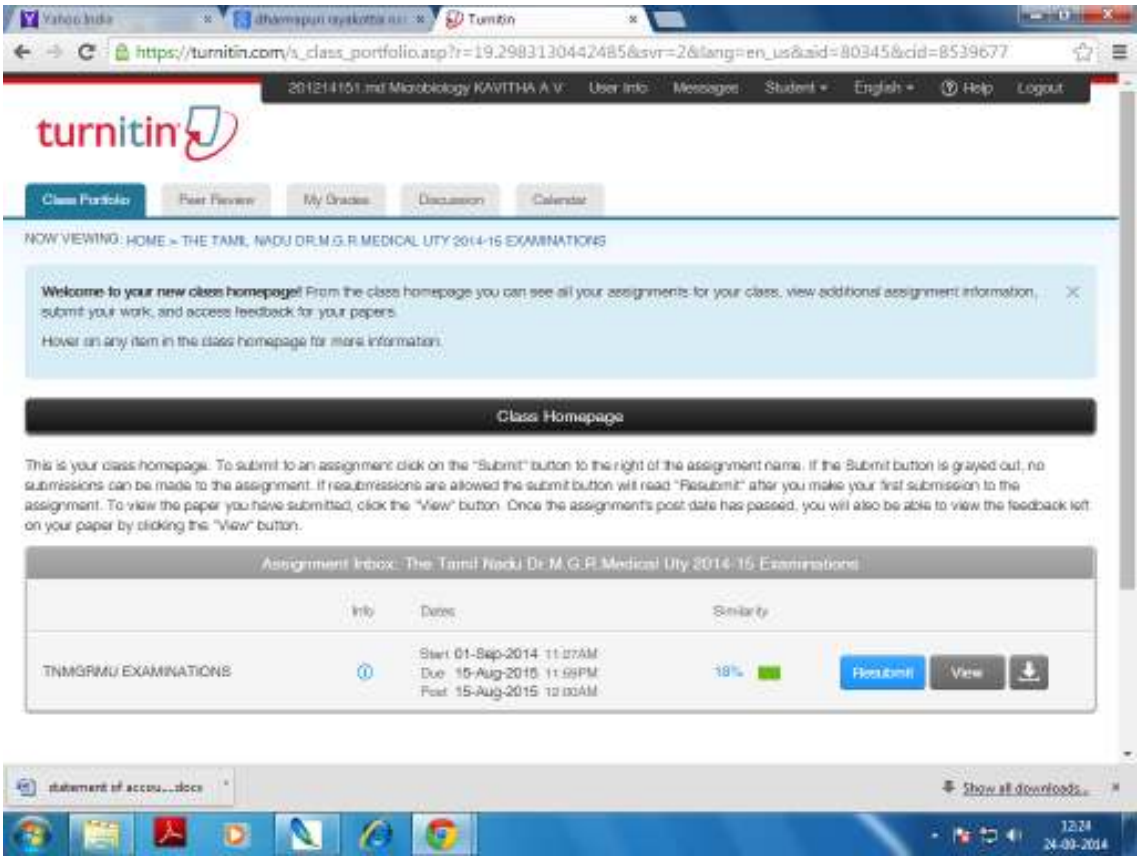
- The present study showed that Gram negative bacilli 60(34.68%) were the predominant bacteria isolated in patients with CSOM.

- Among Gram negative bacilli, Pseudomonas aeruginosa was found to be the most common bacterial pathogen isolated in our study and Staphylococcus aureus was the most common Gram positive organism isolated followed by Klebsiella pneumoniae.

- Among the Enterobacteriaceae isolates, 10(30.30%) were found to be ESBL producers and all of them showed 100% sensitivity to imipenem.

- Among Pseudomonas aeruginosa isolates, 1 (16.66%) was found to be MBL producer. By Ceftazidime – EDTA Test method. Whereas by imipenem –EDTA method none of them were MBL producers.
- Among the Enterobacteriaceae isolates, 9(7.96%) were Amp C producers.
- Among the Staphylococcus aureus isolates, 9(19.53%) were reported to be MRSA producers and all MRSA producers were 100% sensitive to vancomycin and linezolid.

Thus, it is imperative to detect Extended Spectrum Beta Lactamases (ESBL) and Methicillin Resistant Staphylococcus aureus (MRSA) routinely in laboratories by using the various methods mentioned in our study. In CSOM, spread of resistant strains can be prevented by following simple measures like hand washing, appropriate use of broad spectrum antibiotics and early approach to medical care. In cases of middle ear infection, administration of antibiotics should be prescribed cautiously to prevent the emergence of bacterial resistance in the hospital and community.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INTRODUCTION
Chronic suppurative otitis media (CSOM) is one of the most common infections of the middle ear which can cause extra cranial complications and intracranial complications and includes morbidity. It is one of the main causes of preventable hearing loss, principally in the developing world'. CSOM is believed to develop in early childhood, often followed by poorly managed acute otitis media, with its potential spilling into adulthood which accounts for recurrent episodes of chronic discharging ears that can last for many years.
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INSTITUTIONAL ETHICAL COMMITTEE
GOVT.KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Ref.No.10499/ME-1/Ethics/2012 Dt:06.12.2012.
CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A Study on Bacteriological profile in Chronic Suppurative Otitis Media and Antibacterial Susceptibility Pattern and Detection of Methicillin Resistant Staphylococcus Aureus(MRSA) and extended Spectrum Beta lactamases(ESBL) in a Tertiary care Hospital" for dissertation purpose submitted by Dr.A.V.Kavitha, I`st year MD, Microbiology PG Student, Kilpauk Medical College, Chennai

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

13/12/12

CHAIRMAN,
Ethical Committee
Govt.Kilpauk Medical College,Chennai

PROFORMA

NAME:

AGE /SEX:

OP/I.P NUMBER:

ADDRESS:

OCCUPATION:

H/O PRESENT ILLNESS: H/O DM/HT/CAD

DURATION

SYMPTOMS

SIGNS

RISK FACTORS:

PAST HISTORY:

TREATMENT HISTORY:

DIRECT GRAM STAIN

CULTURE

NUTRIENT AGAR

MAC CONKEY AGAR

BLOOD AGAR

ANTIBIOTIC SUSCEPTIBILITY PATTERN

ESBL

Phenotypic Confirmatory Test

MBL

Combined Disc Potentiation Test

AMP C

AmpC Disk Test

Ceftazidime –Imipenem Antagonism Test

MRSA

Cefoxitin Disc Diffusion Test

CONSENT FORM

மய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு

நிறுவனம்/மருத்துவக் கல்லூரி

பங்கு பெறுபவரின் பெயர் :

பங்கு பெறுபவரின் வயது :

பங்கு பெறுபவரின் எண் :

பங்கு பெறுபவர் இதனை (✓) குறிக்கவும்.

மேலே குறிப்பட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது. என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான நிறைந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்கப்பட்டுள்ளது என அறிந்து கொண்டேன். ☐

நான் இவ்வாய்வில் தன்னிச்சையாக நான் பங்கேற்கிறேன், எந்த காரணத்தினாலோ எந்த சட்டரீக்குக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகி கொள்ளலாம் என்றும் அறிந்தும் கொண்டேன். ☐

இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்து மேலும் ஆய்வு மேற்கொள்ளும் பொழுது இந்த ஆய்வில் பங்கு பெறும் மருத்துவர் என்னுடைய மருத்துவ குறிப்புகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். ☐

இந்த ஆய்வில் மூலம் கிடைக்கும் தகவலையோ, முடிவையோ பயன்படுத்திக் கொள்ள மறுக்கமாட்டேன். ☐

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக் கொள்கிறேன். இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உள்ளவுடன் இருப்பேன் என்றும் உறுதியளிக்கிறேன். ☐

பங்கேற்பவரின் கையொப்பம் _____ இடம் _____ தேதி _____

இடம் _____ தேதி _____

பங்கேற்பவரின் பெயர் மற்றும் விலாசம்

சாட்சியாளரின் கையொப்பம்

இடம் _____ தேதி _____

சாட்சியாளரின் பெயர் மற்றும் விலாசம்

ஆய்வாளரின் கையொப்பம்

இடம் _____ தேதி _____

ஆய்வாளரின் பெயர் _____

APPENDIX

GRAM STAIN

Primary stain -crystal violet 10g

Absolute alcohol 100ml Distilled water1litre

Grams iodine-iodine 10g

Potassium iodide 20g Distilled water-1litre

Acetone

Counter stain-dilute carbol fuchsin

1. Flood the crystal violet for one minute
2. Rinse gently with distilled water
3. Flood the slide with grams iodine for one minute
4. Rinse gently with distilled water
5. Decolourise with acetone for only 2-3 secs.
6. Rinse gently with distilled water to remove excess of decolouriser
7. Flood the slide with dilute carbol fuchsin for one minute
8. Rinse the slide with distilled water air dry and examine under oil immersion objective

PEPTONE WATER

Peptone	10g
Sodium chloride	5g
Distilled water	1 litre

Dissolve the ingredients in warm water, adjust the ph to 7.4 -7.5 and filter. Distribute as required and autoclave at 121 degree Celsius for 15 mins.

MacConkey agar:-

This is a useful medium for the cultivation of enterobacteriaceae. It contains a bile salt to inhibit non-intestinal bacteria and lactose with neutral red to distinguish the lactose- fermenting coli forms from the lactose -non-fermenting Salmonella and Shigella groups. The concentration of sodium taurocholate may be reduced to suit less tolerant organisms. The omission of sodium chloride from the medium prevents the spreading of Proteus colonies.

Peptone	20 g
Sodium taurocholate, commercial	5 g
Water	1 litre
Agar	20 g

Neutral red solution, 2% in 50% ethanol	3.5 ml
Lactose, 10% aqueous solution	100 ml

Dissolve the peptone and taurocholate (bile salt) in the water by heating. Add the agar and dissolve it in the steamer or autoclave. If necessary, clear by filtration. Adjust the pH to 7.5. Add the lactose and the neutral red, which should be well shaken before use, and mix. Heat in the autoclave with 'free steam' (c. 100^{o} C) for 1hr., then at 115^{o} C for 15 min. Pour plates.

NUTRIENT AGAR:-	Gm/L
Peptic digest of animal tissue	5.00
Beef extract	1.50
Yeast extract	5.00
Agar	15.00

Dissolve the contents in water and mix by heating Autoclave at 121^{o} C for 15 minutes. Adjust pH to 7.4 + 0.2. Pour 20-25 ml of 9 cm dia. Petri dishes to give 4 mm thickness.

BLOOD AGAR:

Sterile sheep blood	50 ml
Peptone	10 g
Beef extract	3g

Sodium chloride	5 g
Distilled water	1000 ml

Autoclave the nutrient agar base at 121^{o} C for 15 minutes and blood with sterile precautions and distribute in Petri dishes.

MUELLER HINTON AGAR:-

Beef infusion	300 ml
Casein Hydrolysate	17 gm
Starch	1.5 gm
Agar	10 gm
Distilled water	1000 ml

Emulsify the starch in a small amount of cold water, pour into the beef infusion and add the casein-Hydrolysate and the agar. Make up the volume to 1000 ml (1 litre) with distilled water. Dissolve the constituents by heating gently at 100^{o} C with agitation.

Adjust the pH to 7.4. Dispense in screw-capped bottles and sterilize by autoclaving at 121 ^{o} C for 20 minutes. 20 to 25 ml of it is poured into petridishes of 9 cm diameter to give a thickness of 4mm.

McFarland's Turbidity Standard for inoculum preparation

A Barium sulphate 0.5 McFarland standards was prepared as follows

1. A 0.5 ml of 0.048mol/L of Barium chloride was added to 99.5 ml of 0.18 mol/L of H2SO4 with constant stirring to maintain a suspension.
2. Correct density of the turbidity standard was verified by using a spectrophotometer. The absorbance of 625nm should be 0.08 to0.10 for the 0.5 McFarland standards.
3. The Barium sulphate suspension was transferred in 4-6 ml to a screw capped tube of the same size as those used in growing or diluting the bacterial inoculam.
4. These tubes were tightly sealed and stored in the dark at room temperature.
5. The Barium sulphate turbidity standard was vigorously agitated before each use and inspected for a uniform turbid appearance

DIFFERENTIATING CHARACTERS OF ISOLATES COMMONLY AMONG GRAM NEGATIVE BACILLI OBSERVED IN EAR DISCHRGE

Organism	TSI	Citrate	Indole	Oxidase	Catalase	Glucose	Lactose	Sucrose	Maltose	Mannose	Motility
Pseudomonas aeruginosa	K/No change	utilised	-	+	+	-	-	-	+	-	+
Klebsiella pneumoniae	A/A with gas	utilised	-	-	+	+	+	+	+	+	-
Proteus species	A/A with H2S	v	+	-	+	+	-	+	-	-	+
Escherichia coli	A/A with gas	Not utilised	+	-	+	+	+	-	+	+	+

Note: A/A = Acid slant / Acid butt, + = Positive, - = Negative, v - Variable

DIFFERENTIATING CHARACTERS OF ISOLATES COMMONLY OBSERVED IN EAR DISCHARGE AMONG GRAM POSITIVE COCCI

Gram strain	**catalase**	**oxidase**	**hemolysis**	**coagulase**	**Mannitol**	**Bile esculin agar**	**Bacitracin**	**isolate**
Cocci in cluster	positive	negative	±	positive	Positive	negative	resistant	Staphylococcus aureus
Cocci in cluster	positive	negative	±	negative	Negative	negative	resistant	Coagulase negative Staphylococcus aureus
Cocci in pairs	positive	negative	±	negative	Negative	positive	resistant	Enterococci

ZONE SIZE INTERPRETATIVE CHART IN ACCORDING TO NCCLS

Kirby-Bauer Chart

Sl. No.	Drug	Disk Content µg	Resistant mm or less	Intermediate mm	Sensitive mm or more
1	Amoxycillin	10	14 mm	15-16 mm	17 mm
2	Cotrimoxazole	1.25/23.75	10	11-15	16
3	Cephalexin	30	14	15-17	18
4	Ofloxacin	5	12	13-15	16
5	Ciprofloxacin	5	15	16-20	21
6	Gentamicin	10	12	13-14	15
7	Cefotaxime	30	14	15-22	23
8	Ceftazidime	30	14	15-17	18
9	Linezolid	30	-	-	21
10	Amikacin	30	14	15-16	17
11	Imipenem	10	13	15-16	16
12	Piperacillin/tazobactum	100/10	17	18-20	21
13	Vancomycin	30	-	-	15
14	Nitrofurantoin	300	14	15-16	17

ABBREVIATION

MHA	-	Mueller Hinton Agar
CSOM	-	Chronic Suppurative Otitis Media.
CLSI	-	Clinical and Laboratory Standards Institute
ESBL	-	Extended Spectrum Beta Lactamase
E- Test	-	Epsilometer Test
MBL	-	Metallo Beta Lactamase
TEM	-	Temoniera
SHV	-	Sulphy Hydryl Variable
MIC	-	Minimum Inhibition Concentration
EDTA	-	Ethylene Diamine Tetra Acetic Acid.
CLED	-	Cysteine Lactose Electrolyte Deficient
CFU	-	Colony Forming Units.
ATCC	-	American Type Culture Collection

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Sl.No	OP / I.P.No	Age	Sex	income	literacy	Habit of ear cleaning	Direct Stain	Organism	AMX	AMC			CTX						CIP	OF	COT	FT		VAN		CX	IMSA	ESBL	MBL			Follow up
1	14887	24	F	80000	HSC & Above	Y	no pus cells,GPC in clusters	Staphylococcus aureus	S		S	S	S	S		S	R	S	S	S			S	S	R	Y					Recovered
2	14766	17	M	40000	SSC & Below	Y	few pus cells ,GNB seen	Pseudomonas aeruginosa		S			R	S	S	S	S	R	S		S	S			S					Recovered
3	15195	12	F	90000	HSC & Above	N	few pus cells ,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
4	4482	32	M	120000	HSC & Above	N	no pus cells,GNB seen	Pseudomonas aeruginosa					S		R	S	R	S	R		S	S			S					Recovered
								Klebsiella pneumoniae,	S	S			S	S	S	S	R	R	R		S	S			S						
5	15467	29	M	125000	Illiterate	N	few pus cells, GNB seen	Pseudomonas aeruginosa		S			R	S	S	S	S	S	S		S	S			S					Recovered
6	16035	27	M	49000	SSC & Below	Y	few pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
7	16479	38	M	100000	SSC & Below	N	few pus cells ,GNB seen	Pseudomonas aeruginosa		R			R	S	S	R	S	R	S		S	S			S					Recovered

								Proteus mirabilis	R	S			R	S	S	R	R	R	R		S	S								Y	
8	16579	40	F	36000	Illiterate	N	no pus cells,GNB seen	Klebsiella pneumoniae	S	S			S	S	S	S	S	S	S		S	S			R					Recovered
9	399	21	F	90000	SSC & Below	N	no pus cells, GNB seen	Klebsiella pneumoniae	R	S			S	S	S	S	R	S	S		S	S			S					Recovered
10	793	17	F	38000	SSC & Below	Y	no pus cells, GNB seen,GPC in clusters	Pseudomonas aeruginosa		S			R	S	S	S	S	S	S		S	S			S					Recovered
								Staphylococcus aureus	R		R		R	R		S	R	S	S	R			S	S	S					
11	1300875	22	M	67000	HSC & Above	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	R	S	S		S	S			S					LFU
12	130294	28	F	40000	Illiterate	N	few pus cells ,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
13	2205	28	M	65000	HSC & Above	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			R				Y	Recovered
14	2218	27	F	48000	HSC & Above	Y	no pus cells , GPC in pairs	Enterococcus faecalis			R	S				S	R	R	S	S			S	S						Recovered
15	1302869	60	M	55000	Illiterate	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Operated

16	1302687	44	F	35000	SSC & Below	N	no pus cells,no organisms	No growth																							Operated
17	2485	24	F	76000	SSC & Below	N	no pus cells,GNB seen	Klebsiella pneumoniae	S	S			S	S	S	S	S	S	S		S	S			R				Y	Recovered
18	2653	28	F	30000	SSC & Below	Y	few pus cells ,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
19	2459	28	M	40000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
20	2092	21	M	45000	SSC & Below	N	few pus cells ,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
21	3045	57	F	44000	Illiterate	N	no pus cells , GPC in clusters	Staphylococcus aureus	S		S	S	S	S		S	S	R	S	R			S	S	S					Recovered
22	3094	52	M	120000	Illiterate	Y	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
23	3329	30	M	85000	HSC & Above	N	few pus cells ,GNB seen	Pseudomonas aeruginosa		S			S	R	S	S	R	R	S		S	S			R					Recovered
24	1303320	63	F	40000	Illiterate	N	no pus cells, GPC in clusters	Staphylococcus aureus	R		S	S	S	S		S	S	R	R	S			S	S	R	Y				Recovered

25	3647	17	F	45000	SSC & Below	Y	no pus cells,GPC in clusters	Staphylococcus aureus	S		S	S	R	S		S	S	S	S	S			S	S	S					Recovered
26	5302	35	M	90000	HSC & Above	N	no pus cells,GNB seen	Proteus mirabilis	S	S			S	s	S	S	R	S	S		S	S									Recovered
27	5472	37	M	110000	SSC & Below	N	No pus cells, GPC in clusters	Staphylococcus aureus	R		S	S	S	S		S	S	R	S	R			S	S	R	Y					Recovered
28	4247	35	M	46000	HSC & Above	Y	few pus cells,GPC in clusters GPC in pairs and short chains	Staphylococcus aureus	R		S	S	R	S		S	R	S	S	S			S	S	S						Recovered
								Enterococcus faecalis			R	R				S	R	S	S	R			S	S							
29	5730	13	M	48000	SSC & Below	Y	no pus cells, GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	R	R	S		S	S			S						Recovered
30	16163	35	M	90000	Illiterate	N	few pus cells, GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S						Recovered
31	6422	27	M	45000	HSC & Above	N	few pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	R	S	R	S		S	S			S						Recovered
32	117100	26	M	38000	Illiterate	Y	few pus cells ,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S						Recovered
33	13784	8	F	76000	HSC &	Y	no pus	Klebsiella	R	S			R	S	S	R	R	R	R		S	S			S						Recovered

					Above		cells,GNB seen	pneumonia e																								
								Pseudomonas aeruginosa		S			R	S	S	S	S	S	S		S	S			S						
34	13299	52	F	40000	Illiterate	N	few pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	R	S	S	S		S	S			R					Recovered
35	13255	45	F	45000	Illiterate	Y	few pus cells, GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Operated
36	14137	21	F	37000	HSC & Above	Y	no pus cells, GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	R	S		S	S			S					Recovered
37	13881	38	F	68000	SSC & Below	N	no pus cells, GNB seen	Pseudomonas aeruginosa		S			R	S	S	S	S	R	S		S	S			S					Recovered
38	14282	41	F	44000	SSC & Below	Y	no pus cells , GPC seen	Staphylococcus aureus	S		S	S	S	S		S	S	S	R	S			S	S	R	Y				Recovered
39	14489	19	M	65000	HSC & Above	Y	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
40	7886	57	M	48000	Illiterate	N	Few pus cells,GPC in clusters and GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	R	R	R		R	S			R				Y	Recovered
								Staphylococcus aureus	R		R	R	R	R		S	R	S	S				S	S	S					
41	14887	24	F	39000	SSC &	Y		Staphylococ	S		S	S	S	S		S	S	R	S	R			S	S	S					Recovered

					Below			cus aureus																							
42	14907	37	F	78000	SSC & Below	N	few pus cells ,GNB seen	Klebsiella pneumoniae	S	S			S	R	R	S	S	R	S		S	S			S		Y			Recovered
43	15818	10	M	39000	SSC & Below	Y	no pus cells, GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	R	S	S		S	S			S					Recovered
44	11892	32	F	115000	Illiterate	N	no pus cells,GPC in clusters	Staphylococcus aureus	R		S	S	S	S		S	S	R	S	R			S	S	S					Recovered
45	162/12	40	M	125000	HSC & Above	N	no pus cells,gpc in clusters and gpc in pairs seen	Staphylococcus aureus	R		S	S	R	S		S	S	R	S	R			S	S	S					Recovered
								Enterococci faecalis			R	R				R	R	R	S	S			S	S						
46	348	32	M	44000	HSC & Above	Y	no pus cells, GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
47	1481	18	F	39000	HSC & Above	Y	no pus cells,no organisms	No growth																						LFU
48	1302694	65	M	45000	Illiterate	Y	no pus cells,no organisms	No growth																						Recovered
49	1411	62	M	40000	Illiterate	N	few pus cells ,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered

50	6058	30	M	38000	Illiterate	N	no pus cells,no organisms	No growth																							LFU
51	86/13	65	M	45000	Illiterate	N	no pus cells,no organisms	No growth																							Recovered
52	1304534	8	F	47000	SSC & Below	Y	No pus cells, GPC in pairs and short cains	Enterococcus faecalis			S	S				S	S	R	R	R			S	S						Recovered
53	1305831	61	F	58000	Illiterate	Y	no pus cells,no organisms	No growth																							Operated
54	1306073	70	M	39000	Illiterate	N	no pus cells,no organisms	No growth																							Recovered
55	1313456	52	F	35000	Illiterate	N	no pus cells, no organisms	No growth																							LFU
56	13341	33	F	90000	Illiterate	N	no pus cells ,no organism	No growth																							Recovered
57	3847	17	F	39000	HSC & Above	N	few pus cells ,no organism	No growth																							Recovered
58	1314379	13	M	75000	SSC & Below	Y	no pus cells ,no organism	No growth																							LFU
59	1310632	46	F	45000	SSC & Below	N	no pus cells,no organisms	No growth																							Recovered
60	11646	15	M	40000	SSC &	Y	no pus	Proteus	R	S			S	S	S	S	S	S	S		S	S									Recovered

					Below		cells,GNB seen	mirabilis																								
61	14687	20	F	80000	HSC & Above	N	no pus cells,no organisms	No growth																								Recovered
62	13341	33	F	60000	Illiterate	Y	no pus cells,no organisms	No growth																								Recovered
63	13239	32	F	45000	SSC & Below	Y	no pus cells,no organisms	No growth																								LFU
64	13788	35	M	40000	SSC & Below	N	no pus cells,GNB seen	Klebsiella pneumoniae	R	S			S	R	R	S	S	S	S		s	s			S		Y				Operated
65	14078	26	M	35000	HSC & Above	N	no pus cells,no organisms	No growth																								Recovered
66	16194	42	M	110000	SSC & Below	Y	no pus cells,GPC in clusters	Staphylococcus aureus	S		S	S	S	S		S	S	S	S	R			S	S	R	Y					Recovered
67	13015	17	F	40000	SSC & Below	N	no pus cells,no organisms	No growth															S	S							Recovered
68	1638	13	M	38000	SSC & Below	Y	no pus cells , no organisms	No growth																								LFU
69	176/13	39	M	45000	SSC &	N	no pus cells,GNB	Pseudomonas		R			R	S	S	S	R	S	S		S	S			S						Recovered

					Below		seen	aeruginosa																								
70	18892	29	M	120000	Illiterate	N	no pus cells,GNB seen	Pseudomonas aeruginosa		R			S	S	S	S	S	S	S		S	S			S					Recovered
71	3827	20	M	40000	HSC & Above	Y	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	R	S		S	S			S					Recovered
72	2567	27	F	100000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		R			R	S	S	S	R	S	S		S	S			S					Recovered
73	17729	29	F	48000	Illiterate	Y	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	R	S	S		S	S			S					Recovered
74	13829	22	M	46000	SSC & Below	N	few pus cells,GPC in clusterss	Staphylococcus aureus			R	R	S	S		R	R	R	S	S			S	S	S					Recovered
75	19563	19	M	38000	HSC & Above	N	no pus cells,GPC in clusterss	Staphylococcus aureus	S		S	S	S	S		S	S	S	S	S			S	S	R	Y					Recovered
76	19707	31	F	115000	SSC & Below	Y	few pus cells,GNB seen	Proteus mirabilis	S	S			S	S	S	S	R	S	S		S	S								Recovered
77	19428	12	M	45000	SSC & Below	Y	no pus cells,GPC in clusterss	Staphylococcus aureus	R		S	S	S	S		R	S	R	S	S			S	S	S					Recovered
78	19428	13	M	40000	SSC & Below	N	Few pus cells,GPC in clusters .	Staphylococcus aureus	R		R	R	S	S		S	S	S	R	S			S	S	R	Y					Recovered

79	19946	6	F	60000	HSC & Above	Y	no pus cells,GNB seen	Klebsiella pneumoniae	R	R			R	S	S	S	R	S	R		S	S			R				Y	Recovered
								Staphylococcus aureus	R		R	R	R	R		S	R	R	S	R			S	S	S					
80	1333264	13	M	46000	HSC & Above	N	no pus cells,GNB seen	Pseudomonas aeruginosa		R			S	S	S	S	R	S	S		S	S			S					Recovered
81	13332	13	M	37000	HSC & Above	Y	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	R	S		S	S			S					Recovered
82	10260	40	F	78000	Illiterate	N	no pus cells ,no organisms	No growth																							Recovered
83	13546	22	F	45000	SSC & Below	N	no pus cells, GPC in clusters seen	Staphylococcus aureus	S		S	R	S	S		S	S	R	R	R			S	S	S					Recovered
84	13789	50	M	55000	Illiterate	N	no pus cells, GPC in clusters	Staphylococcus aureus	R		S	S	R	S		S	R	S	S	R			S	S	R	Y				Recovered
85	4351	33	F	40000	Illiterate	Y	no pus cells, GNB seen	Klebsiella pneumoniae	S	S			S	S	S	S	S	S	S		S	S			S					LFU
86	11543	25	M	72000	SSC & Below	Y	no pus cells, GPC in clusters	CONS	S		S	S	S	S		S	S	S	R	S			S	S	S					Recovered
87	2167	22	F	45000	SSC & Below	Y	no pus cells, GNB seen	Klebsiella pneumoniae	R	S			S	R	R	S	S	R	S		S	S			S		Y			Operated

88	1325	39	F	47000	SSC & Below	N	no pus cells, no organisms	No growth																							Recovered
89	111678	54	M	60000	Illiterate	N	no pus cells, GPC in clusters	Staphylococcus aureus	R		S	S	S	S		S	S	S	S	S			S	S	S					Recovered
90	1657	34	F	45000	SSC & Below	N	no pus cells, no organisms	No growth																							LFU
91	1325	39	M	65000	SSC & Below	N	no pus cells, no organisms	No growth																							Recovered
92	1456	52	M	40000	Illiterate	N	few pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
93	1298	55	M	80000	Illiterate	N	no pus cells, no organisms	No growth																							Recovered
94	16785	35	F	45000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	R	R	S		S	S			S					Recovered
95	1402706	39	F	80000	SSC & Below	N	no pus cells, no organisms	No growth																							Recovered
96	2425	37	F	38000	Illiterate	Y	few pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	R	S	S		S	S			S					Recovered
97	2410	65	M	100000	Illiterate	N	few pus cells,GNB seen	Klebsiella pneumoniae	S	R			S	R	R	R	R	S	S		S	S			S		Y			Operated
98	2450	17	F	46000	SSC & Below	N	few pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered

99	2131	45	F	60000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	R	R	S	R	S	S		S	S			R				Y	Recovered
100	1339462	48	M	45000	Illiterate	N	no pus cells, no organisms	No growth																							Recovered
101	2556	9	M	50000	HSC & Above	N	no pus cells, GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
102	2762	22	F	48000	HSC & Above	N	no pus cells, GPC in clusters	Staphylococcus aureus	S		S	S	R	S		S	S	S	S	S			S	S	R	Y				Recovered
103	2688	37	M	110000	HSC & Above	Y	no pus cells, no organisms	No growth																							Recovered
104	731	7	M	80000	SSC & Below	N	no pus cells, GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	R	S	S		S	S			S					Recovered
105	2775	45	F	45000	SSC & Below	N	no pus cells, no organisms	No growth																							Recovered
106	2734	40	M	105000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	R	S		S	S			S					Recovered
107	826	38	F	40000	Illiterate	N	no pus cells,GPC in clusters	Staphylococcus aureus	R		S	S	S	S		S	S	S	S	S			S	S	S					Recovered
108	5042	32	F	38000	SSC & Below	N	no pus cells, GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	R	S		S	S			S					Recovered
109	545	32	F	42000	SSC &	N	No pus cells,	Enterococcu			S	S				S	S	S	S	R			S	S						Operated

					Below		GPC in pairs	s faecalis																							
110	269	23	M	40000	HSC & Above	N	no pus cells,GPC in clusters	Staphylococcus aureus	R		S	S	S	S		S	S	R	S	R			S	S	S					Recovered
111	2285	55	F	55000	Illiterate	N	few pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
112	1567	49	M	36000	Illiterate	N	no pus cells,GPC in clusterss	CONS	S		S	S	S	S		S	S	S	S	S			S	S	R					Recovered
113	11341	56	M	130000	SSC & Below	N	few pus cells,GNB seen	Pseudomonas aeruginosa		S			S	R	R	S	S	S	S		S	S			S			Y		Operated
114	2415	30	F	45000	HSC & Above	N	no pus cells, no organisms	No growth																						Recovered
115	3395	33	M	110000	SSC & Below	Y	few pus cells,GPC in clusterss	Staphylococcus aureus	S		S	S	S	R		S	S	R	S	R			S	S	S					Recovered
116	126	58	M	38000	Illiterate	Y	few pus cells,GNB seen	Pseudomonas aeruginosa		S			R	S	S	S	S	S	S		S	S			S					Recovered
117	4204	45	M	40000	HSC & Above	N	few pus cells,GPC in clusterss	Staphylococcus aureus			S	S	S	S		S	S	S	S	R			S	S	S					Recovered
118	4209	53	F	45000	Illiterate	N	few pus cells,GNB seen	Klebsiella pneumoniae	S	S			S	R	R	S	S	S	S		S	S			S		Y			Recovered
119	4077	25	F	38000	HSC &	Y	no pus cells,GNB	Pseudomonas		S			S	S	S	S	R	R	S		S	S			S					LFU

					Above		seen	aeruginosa																								
120	4401	37	F	75000	SSC & Below	Y	no pus cells,GNB seen	Klebsiella pneumoniae	R	S			R	R	R	S	S	R	S		S	S			S		Y			Recovered
121	4493	12	M	42000	HSC & Above	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			R	S	S	S	S	S	S		S	S			S					Recovered
122	3392	12	F	40000	HSC & Above	N	few pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
123	1405787	55	M	130000	Illiterate	N	few pus cells,GNB seen	Klebsiella pneumoniae	R	S			S	R	R	R	S	S	S		S	S			S		Y			Recovered
124	1406240	2	M	35000	HSC & Above	N	no pus cells, no organisms	No growth																						Recovered
125	32	34	F	49000	Illiterate	N	no pus cells, no organisms	No growth																						Recovered
126	1405787	62	M	43000	Illiterate	N	no pus cells,GNB seen	Klebsiella pneumoniae	R	S			S	S	S	S	S	S	S		S	S			S					Operated
127	4537	34	M	45000	Illiterate	Y	no pus cells,no organisms	No growth																						LFU
128	5087	20	M	39000	HSC & Above	N	few pus cells,GNB	Pseudomonas		S			S	S	S	S	S	S	S		S	S			S					Recovered

							seen	aeruginosa																							
129	5209	65	M	40000	Illiterate	N	no pus cells,no organisms	No growth																						Recovered
130	1408	49	F	38000	Illiterate	Y	few pus cells,no organisms	No growth																						Recovered
131	5645	56	F	65000	SSC & Below	N	few pus cells,GNB seen, GPC in clusters	Pseudomonas aeruginosa		S			S	S	S	S	S	R	R		S	S			S					Recovered
								Staphylococcus aureus	R		R	R	R	S		R	R	R	R	R			S	S	S					
132	5676	45	F	42000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		R			S	S	S	S	S	S	S		S	S			S					Recovered
133	1408/99	35	M	75000	SSC & Below	N	no pus cells,GPC in clusterss	Staphylococcus aureus	S		S	S	S	S		S	R	R	S	R			S	S	S					Operated
134	4376	45	F	42000	SSC & Below	N	no pus cells,GPC in clusterss	CONS	S		S	S	S	S		S	S	S	S	S			S	S	S					Recovered
135	5262	27	F	80000	Illiterate	N	no pus cells,no organisms	No growth																						Recovered
136	2288	40	F	45000	Illiterate	N	no pus cells, no organisms	No growth																						Recovered
137	1410773	55	M	50000	SSC &	N	no pus cells,GNB	Klebsiella pneumonia	R	S			S	S	S	S	S	S	S		S	S			S					Operated

					Below		seen	e																							
138	1410769	29	F	43000	SSC & Below	Y	few pus cells,GNB seen	Proteus mirabilis	R	S			S	S	S	S	S	R	S		S	S							Operated
139	5994	54	F	60000	Illiterate	Y	no pus cells,GNB seen	Pseudomonas aeruginosa		S			R	S	S	S	S	S	S		S	S			S				Recovered
								Klebsiella pneumoniae	R	R			R	S	S	R	R	R	R		S	S			S				
140	6188	30	F	40000	SSC & Below	N	no pus cells,GPC in clusterss	Staphylococcus aureus	R		S	S	S	S		S	S	S	S	S			S	S	S				Recovered
141	6278	41	F	35000	Illiterate	N	no pus cells,GPC in clusterss	Staphylococcus aureus	R		S	R	S	S		S	S	S	S	R			S	S	S				Recovered
142	5638	43	M	75000	SSC & Below	N	no pus cells, no organisms	No growth																					LFU
143	1390	13	M	38000	SSC & Below	N	no pus cells,no organisms	No growth																					Recovered
144	6436	43	M	80000	SSC & Below	Y	no pus cells,no organisms	No growth																					Recovered
145	13829	29	M	35000	HSC &	N	no pus cells,GPC in	Staphylococ	R		S	S	S	S		S	R	S	S	S			S	S	S				Recovered

					Above		clusters	cus aureus																							
146	2051	28	F	42000	SSC & Below	N	no pus cells,no organisms	No growth																						LFU
147	1413620	45	F	47000	SSC & Below	N	few pus cells ,GNB seen	Klebsiella pneumoniae	R	S			R	S	S	S	S	R	S		S	S			S					Recovered
148	7516	5	F	39000	HSC & Above	N	no pus cells, GPC in clusters seen	CONS	R		S	S	R	S		S	R	S	S	R			S	S	S					Recovered
149	8129	28	F	60000	SSC & Below	Y	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					LFU
150	7668	39	F	76000	SSC & Below	N	no pus cells, GPC in clusters seen	CONS	R		R	S	S	S		S	S	R	S	R			S	S	S					Recovered
151	2357	9	M	38000	HSC & Above	Y	No pus cells, GPC in clusters seen, GNB seen	Staphylococcus aureus	R		S	R	R	S		S	R	S	S	R			S	S	S					Recovered
								Proteus mirabilis	R	S			R	S	S	S	R	R	S		S	S								
152	1417650	73	M	75000	Illiterate	N	No pus cells, GPC in clusters	CONS	R		S	R	S	S		S	S	S	S	R			S	S	S					Operated
153	9001	42	F	46000	SSC & Below	N	No pus cells, GPC in clusters	Staphylococcus aureus	R		S	R	S	S		S	S	S	S	R			S	S	S					Recovered
154	3013	30	M	80000	Illiterate	N	no pus cells,	No growth																						Recovered

							no organisms																							
155	8148	24	M	90000	SSC & Below	N	No pus cells, GPC in clusters	Staphylococcus aureus	R		S	S	S	S		S	S	S	S	S			S	S	S					Recovered
156	8150	60	M	42000	Illiterate	N	no pus cells, GNB seen	Klebsiella pneumoniae	R	S			R	S	S	S	S	S	S		S	S			S					Recovered
157	7890	27	F	38000	SSC & Below	N	Few pus cells,GPC in clusters and GNB seen	Pseudomonas aeruginosa		S			R	S	R	S	S	S	S		S	S			S					Recovered
								Staphylococcus aureus	R		R	R	R	R		R	R	R	R				S	S	S					
158	7104	50	M	75000	SSC & Below	Y	no pus cells,no organisms	No growth																						Recovered
159	4560	23	M	43000	HSC & Above	N	No pus cells, GPC in clusters	Staphylococcus aureus	R		R	S	S	S		S	S	S	S	R			S	S	S					Recovered
160	1417494	49	M	45000	Illiterate	N	No pus cells,GPC in clusters	Staphylococcus aureus	R		S	S	S	S		R	R	S	S	R			S	S	S					Operated
161	1417898	61	M	39000	SSC & Below	N	No pus cells,GPC in clusters	Staphylococcus aureus	R		R	S	R	S		S	S	S	S	R			S	S	S					Operated
162	5334	45	M	68000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	R	R	S		S	S			S					Recovered

163	10580	25	M	85000	HSC & Above	N	No pus clls, GNB seen	Proteus mirabilis	R	R			R	S	S	S	S	R	S		S	S								Recovered
164	9072	7	M	43000	SSC & Below	Y	no pus cells, no organisms	No growth		S																					Recovered
165	10754	30	M	40000	SSC & Below	N	No pus cells, GPC in clusters	Staphylococcus aureus	R		S	S	S	S		S	S	S	S	R			S	S	S					LFU
166	1410967	30	F	58000	Illiterate	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Operated
167	9794	14	M	75000	SSC & Below	N	few pus cells ,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
								Klebsiella pneumoniae	R	S			R	S	S	S	R	R	R		S	S			S					
168	10924	32	F	35000	SSC & Below	Y	no pus cells, GPC in clusters	Staphylococcus aureus	R		S	S	S	S		S	S	R	S	R			S	S	S					Recovered
169	1419695	41	M	38000	HSC & Above	N	no pus cells . GPC in clusters	CONS	R		R	S	S	S		S	S	R	S	S			S	S	S					Operated
170	110/14	59	M	55000	HSC & Above	N	few pus cells ,GNB seen	Escherichia coli						S							S	S								Recovered
171	11076	21	F	40000	SSC &	N	no pus cells,	No growth																							LFU

					Below		no organisms																							
172	11850	20	F	45000	SSC & Below	N	few pus cells, no organisms	No growth																						Recovered
173	15530	16	F	70000	SSC & Below	N	no pus cells, no organisms	No growth																						Recovered
174	11626	72	M	46000	Illiterate	N	no pus cells, no organisms	No growth																						Recovered
175	11094	10	M	66000	HSC & Above	N	no pus cells, GNB seen	Pseudomonas aeruginosa		S			R	S	S	S	S	S	S		S	S			S					LFU
176	1486	38	M	47000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					LFU
177	12244	23	F	75000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
178	11536	16	F	38000	Illiterate	Y	no pus cells,no organisms	No growth							S															LFU
179	17617	21	F	36000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			S	S	S	S	S	S	S		S	S			S					Recovered
180	1417468	32	M	80000	HSC & Above	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S				S	S	S	S	S	S		S	S			S					Operated
181	9653	43	M	40000	HSC & Above	N	no pus cells,GPC in clusters	Staphylococcus aureus	R		R	S	S	S		S	S	S	S	S			S	S	S					Recovered

182	8940	24	F	36000	SSC & Below	N	no pus cells,no organisms	No growth																							Recovered
183	13188	32	M	60000	SSC & Below	N	no pus cells ,short GNB seen	Klebsiella pneumoniae	R	R			R	S	S	S	S	R	S		S	S			S					Operated
184	13299	53	F	50000	Illiterate	N	few pus cells,GNB seen	Pseudomonas aeruginosa		S			R	S	S	S	S	S	S		S	S			S					Recovered
185	20774	30	F	40000	Illiterate	N	no pus cells, no organisms	No growth																							Recovered
186	3246	34	F	67000	SSC & Below	N	pus cells ,GNB seen	Pseudomonas aeruginosa		S			R	S	S	S	R	S	S		S	S			S					Recovered
187	4564	40	F	43000	SSC & Below	N	few pus cells, GNB seen	Klebsiella pneumoniae	R	R			R	R	R	S	R	R	R		s	s			s		Y			Recovered
188	4875	38	F	40000	SSC & Below	N	few pus cells,GNB seen	Pseudomonas aeruginosa		S			R	S	S	S	S	R	S		S	S			S					Recovered
189	1322001	37	F	85000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		S			R	S	S	S	S	S	S		S	S			S					Recovered
190	2390	28	M	95000	HSC & Above	N	few pus cells,GNB seen	Pseudomonas aeruginosa		R			R	S	S	S	S	R	S		S	S			S					LFU
191	17185	13	M	40000	HSC & Above	N	no pus cells,GPC in clusterss	CONS	R		R	S	R	s		S	R	R	S	R			S	S	R					Recovered

192	2378	35	F	55000	SSC & Below	N	few pus cells,GNB seen	Pseudomonas aeruginosa		R			R	S	S	S	S	S	S		S	S			S					Recovered
193	14168	65	F	45000	Illiterate	N	few pus cells,GNB seen	Pseudomonas aeruginosa		R			R	S	S	S	R	R	S		S	S			R				Y	LFU
194	26846	45	M	90000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		R			R	R	R	S	R	R	S		S	S			R				Y	Recovered
195	4139	19	M	60000	HSC & Above	N	few pus cells,GNB seen, no pus cells, GPC in clusterss	Klebsiella pneumoniae	R	R			R	R	R	R	R	R	S		S	S			S		Y			Recovered
								CONS	R		R	R	R	R		R	R	R	R	R			S	S	S						
196	162/12	40	M	46000	SSC & Below	Y	no pus cells,GPC in clusters	Staphylococcus aureus	R		R	S	S	S		S	S	S	S	R			S	S	S					Recovered
197	11892	32	F	40000	SSC & Below	N	no pus cells,GPC in clusters	Staphylococcus aureus	R		R	S	S	S		S	R	S	S	S			S	S	S					Recovered
198	15818	10	M	60000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		R			R	S	S	S	S	R	S		S	S			S					Recovered
199	15295	17	F	38000	SSC & Below	N	no pus cells,no organisms	No growth																							Recovered
200	14907	37	F	70000	SSC & Below	no	no pus cells,GNB seen	Klebsiella pneumoniae	R	R			R	R	R	S	R	R	S		S	S			S		Y			Recovered

201	14078	26	M	55000	SSC & Below	N	no pus cells,no organisms	No growth																						Recovered
202	14766	17	M	46000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa		R			R	S	S	S	S	S	S		S	S			S					Recovered
203	7886	57	M	42000	Illiterate	N	Few pus cells,GPC in clusters and GNB seen	Pseudomonas aeruginosa		R			R	S	S	S	S	S	R		S	S	S	S	S					LFU
								Staphylococcus aureus	R		R	R	R	R		S	R	S	S	S			S	S	S					
204	5326	28	F	75000	SSC & Below	N	no pus cells,GNB seen	Pseudomonas aeruginosa					R	S	S	S	S	S	S		S	S			S					Recovered
205	12764	30	F	48000	SSC & Below	N	no pus cells,no organisms	No growth																						Recovered
206	13230	41	M	80000	Illiterate	N	no pus cells ,GNB seen	Pseudomonas aeruginosa,		R			R	S	S	S	R	S	S		S	S			S					Recovered
207	1307691	12	M	75000	SSC & Below	N	few pus cells ,GNB seen	Klebsiella pneumoniae	R	R			R	S	S	S	R	R	R		S	S			R				Y	Recovered
								Pseudomonas aeruginosa		R			R	R	R	S	R	R	S		R	S			R				Y	
208	13334	7	M	40000	HSC & Above	N	no pus cells, no organisms	No growth																						Recovered

209	16194	42	M	68000	SSC & Below	N	no pus cells,GPC in clusters	Staphylococcus aureus	R		R	S	S	S		S	R	R	S	S			S	S	S					Recovered
210	4780	30	M	80000	SSC & Below	Y	no pus cells, GPC in clusters seen	Staphylococcus aureus	R		R	S	S	S		S	R	R	S	S			S	S	S					Recovered
211	1307786	27	F	41000	SSC & Below	Y	no pus cells, no organisms	No growth																						LFU
212	18800	29	F	36000	SSC & Below	N	few pus cell,s no organisms	No growth																						Recovered